EXPERIMENT STATION
OF THE
HAWAIIAN SUGAR PLANTERS' ASSOCIATION

Red-Stripe Disease Studies

By
The Staff of the
Department of Pathology

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LETTER OF TRANSMITTAL

To the Experiment Station Committee of the
Hawaiian Sugar Planters' Association,
Honolulu, T. H.

Gentlemen:

I transmit herewith for publication a paper on Red-Stripe Disease Studies by the staff of the Department of Pathology.

Yours very truly,

H. P. AGEE,
Director.

Honolulu, T. H.,
September 9, 1925.

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The Cause of Red-Stripe Disease of Sugar Cane

By H. Atherton Lee and J. P. Martin

INTRODUCTION

In a previous publication,[1] red-stripe disease of the Yellow Tip, Red Tip and Striped Tip varieties of sugar cane has been described and statements made concerning the association of a bacterial organism with the disease. The present paper will present briefly the isolation and inoculation studies which prove the causal relationship of this organism to the disease.

Red-stripe disease, to the present time, occurs only in the Kohala district of the island of Hawaii. Since no equipment for histological or isolation studies existed in Kohala, a considerable handicap was placed on the studies, not only in getting fresh material to Honolulu, but also because extreme precautions were necessary to prevent the spread of the disease from the pathology laboratory to the cane fields on Oahu. All material taken to Honolulu was placed in galvanized iron boxes in Kohala and the joints of the boxes sealed with adhesive tape. These boxes were opened only in the isolation chamber of the pathology laboratories, and material after being used was removed immediately and autoclaved. All studies of the disease have been confined to the culture chamber of the laboratory and every precaution has been exerted to prevent the disease organism escaping from this chamber.

Examination of the Diseased Tissues

Hand sections were made of lesions of the disease brought from Kohala on February 28, 1924. It was readily evident under the microscope that bacteria were associated with the reddened stripes; large masses of motile bacteria oozed out from the sections. The examination was repeated a number of times and the bacteria were readily found and constantly associated with the watery, dark-green limits of the lesions.

Ten series of glucose-agar dilution plates were made from this first material brought from Kohala. On all plantings bacteria showed up, at first apparently uniformly. The most abundant colonies were at first pearly white, but after five or six days they gradually became mustard yellow (R).[2] With the lapse of time it also became evident that there were colonies of two distinct organisms on these plates; the larger number turned yellow, but smaller, smooth, shiny colonies showed up more slowly of a whitish color which did not become yellow with time. Both types of colonies were transferred to glucose-agar slants. For con-

[1]Lee, H. Atherton, and Jennings, W. C., Bacterial red-stripe disease of Tip canes. Hawaiian Sugar Planters' Association, Experiment Station, Circular 42, April, 1924.

[2]R. refers to Ridgway, Robert. Color standards and color nomenclature, Washington, D. C., 1917.

venience the organism forming yellow colonies was termed Organism A and that forming whitish colonies was called Organism B; a third white organism, quite evidently distinct from B which occurred only occasionally in the plates, was termed Organism X. A second strain of the organism forming yellow colonies was called Organism C.

There had been some previous work on the disease associating fungi with the trouble, so that two fungi obtained from plantings on separate plates were maintained in culture and labeled Fungus D and Fungus E.

Inoculation Studies

The above cultures A, B, X, C, D and E were taken to Kohala and preliminary inoculations made from each. It may be said that we were very confident that Organism A, which formed yellow colonies on agar and was so abundant in the plates, was the pathogene; the other cultures were used for inoculations in order to overlook no organisms with possible pathogenic relationship to the disease, and with the view also that the non-pathogenes would act as controls on the pathogene.

The methods of inoculation of all organisms were identical. A sterile needle was used to transfer the organism to be tested from the culture to the cane leaf. Two leaves on each plant were inoculated; the youngest leaf on each plant just emerging from the central cylinder, and a middle-aged leaf, usually the third from the youngest leaf. The inoculum was placed on the leaf and scratched into the surface with the needle and two or three punctures made in the same area where the scratches were made. Five inoculations were made diagonally across each leaf. After placing moist cotton at the bases of the leaves, both inoculated leaves of the plant were wrapped in paraffin paper and the whole covered with opaque paper to prevent burning with the sun. The cane plants used for the inoculations were Striped Tip ratoons in a small field where no red stripe had been observed previously. The cane was five to six feet high and in moderately good growing condition.

Table I

Showing Inoculations Made With Organisms Isolated from Red-Stripe Disease and Results. The Inoculations Were Made March 14, 1924, and Results Observed May 2, 1924.

Stalk Number	Organism	Positive results on old leaves		Positive results on young leaves	
		Number	Per cent	Number	Per cent
1	D	0	0	0	0
2		0	0	0	0
3		0	0	0	0
4		0	0	0	0
5		0	0	0	0
6		0	0	0	0
7		0	0	0	0
8		0	0	0	0
9		0	0	0	0

TABLE I (Concluded)

Stalk Number	Organism	Positive results on old leaves Number	Positive results on old leaves Per cent	Positive results on young leaves Number	Positive results on young leaves Per cent
10		0	0	0	0
11	E	0	0	0	0
12		0	0	0	0
13		0	0	0	0
14		0	0	0	0
15		0	0	0	0
16		0	0	0	0
17		0	0	0	0
18		0	0	0	0
19		0	0	0	0
20		0	0	0	0
21	X	0	0	0	0
22		0	0	0	0
23		0	0	0	0
24		0	0	0	0
25		0	0	0	0
26		0	0	0	0
27		0	0	0	0
28		0	0	0	0
29		0	0	0	0
30		0	0	0	0
31	B	negative	–	negative	–
32		negative	–	positive*	–
33		positive*	–	positive	–
34		negative	–	positive	–
35		positive	–	positive	–
36		negative	–	negative	–
37		negative	–	negative	–
38		positive	–	positive	–
39		positive	–	positive	–
40		positive	–	positive	–
41	A	positive*	–	positive	–
42		negative	–	negative	–
43		positive	–	negative	–
44		negative	–	negative	–
45		negative	–	negative	–
46		negative	–	negative	–
47		positive	–	negative	–
48		negative	–	negative	–
49		negative	–	negative	–
50		negative	–	negative	–
51	C	0	0	0	0
52		0	0	0	0
53		0	0	0	0
54		0	0	0	0
55		0	0	0	0
56		0	0	0	0
57		0	0	0	0
58		0	0	0	0
59		0	0	0	0
60		0	0	0	0

The results obtained from these first series of inoculations were surprising in that they indicated the whitish Organism B as the pathogene, while Organism A and the second strain, which we called Organism C, occurring so uniformly in the isolated plates, were almost entirely negative. On looking back over the results we account for the positive results on plants 41,

* Results of this series were only observed as to whether the leaf was positive or negative; no count was made of the results at the individual inoculations.

43 and 47 inoculated with Organism A, as a carry-over from the series of inoculations with Organism B, which had just been completed.

The inoculations showing positive results were typical cases of red-stripe disease. The first symptom was the development of a dark, watery, green stripe, 1 to 1½ millimeters in width, visible on both surfaces of the leaf, which advanced rapidly, longitudinally, and gradually developed a red color following the advance of the watery green area. Many of the inoculations developed into highly virulent cases of the disease, in sharp contrast to the results with the other organisms and the comparative freedom from the disease of the rest of the field.

While in Kohala further inoculations were made by the same methods on Striped Tip ratoons in the same field, using only Organisms A and B and a series of twenty inoculated leaves with needle punctures with no inoculum as controls. Both Organisms A and B had been replated in order to avoid any possibility of working with mixed cultures.

The inoculations on plants 106-110 inclusive, were made without punctures of any kind in order to determine the possibility of infection at stomata or mechanical breaks. Portions of cultures of Organism B were taken from the tubes with a sterile scalpel and placed upon the innermost leaves of the central cylinder of each stalk. Moistened cotton was inserted about the young leaves and the entire stalk was wrapped in paraffin paper, covered with opaque paper, tied top and bottom, and labeled.

Ten days after the inoculations were made, the following telegram was received in Honolulu from W. C. Jennings at Kohala:

> Kohala, April 14, 1924.
>
> "Six each series inoculations examined stop B all positive A and checks all negative. Jennings."

In the meantime isolations had been made in Honolulu from the positive results with Organism B on plants 31 to 40, and also from the positive result on plant 43 with Organism A. The whitish Organism B was recovered in culture with no difficulty from several of plants 31 to 40 and from plant 43. Fourteen days after the receipt of the telegram, the final examination of the whole of the second series of inoculations was made. The results in detail are shown in Table II.

TABLE II

Showing Results of Inoculations With Organisms A and B and Control Punctures With No Inoculum. The Inoculations Were Made April 4, 1924, and Results Observed April 28, 1924.

Stalk Number	Organism	Positive results on old leaves		Positive results on young leaves	
		Number	Per cent	Number	Per cent
61	control	0	0	0	0
62	"	0	0	0	0
63	"	0	0	0	0
64	"	0	0	0	0
65	"	0	0	0	0
66	"	0	0	0	0
67	"	0	0	0	0
68	"	0	0	0	0
69	"	0	0	0	0
70	"	0	0	0	0
71	A	3	60	3	60*
72	A	1	20	0	0*
73	A	3	60	2	40*
74	A	0	0	1	20*
75	A	0	0	1	20*
76	A	3	60	2	40*
77	A	0	0	0	0
78	A	0	0	0	0
79	A	0	0	1	20†
80	A	0	0	0	0
81	A	0	0	0	0
82	A	0	0	0	0
83	A	lost	0	0	0
84	A	0	0	0	0
85	A	0	0	0	0
86	A	0	0	0	0
87	A	0	0	0	0
88	A	0	0	0	0
89	A	0	0	0	0
90	A	0	0	0	0
91	A	0	0	0	0
92	A	0	0	0	0
93	A	0	0	0	0
94	A	0	0	0	0
95	A	0	0	0	0
96	B	2	40	2	40
97	B	1	20	3	60
98	B	2	40	1	20
99	B	3	60	1	20
100	B	3	60	3	60
101	B	2	40	3	60
102	B	1	20	2	40
103	B	1	20	1	60
104	B	2	40	lost	–
105	B	1	20	3	60
106	B	positive	–	–	–
107	B	"	–	–	–
108	B	"	–	–	–
109	B	"	–	–	–
110	B	"	–	–	–

The results with Organism A of this second series of inoculations were confusing until fully tabulated. It then became apparent that the six plants inoculated with Organism A, which showed positive results, were those

* First 6 plants examined by Mr. Jennings.
† A new lesion, apparently chance.

examined two weeks previously by Mr. Jennings. The results are so clear cut as to leave little doubt of the infection of these six plants at the time of being unwrapped and inspected during the first examination for results, on April 14, especially in view of the fact that they were negative at the time of this examination. This peculiar circumstance also points to the easy method of spread and the highly infectious nature of the disease. In this series Organism B gave 100 per cent positive results for the number of leaves inoculated, and from the standpoint of individual inoculations gave 37 per cent positive results.

Organism B, which had been reisolated in Honolulu, was now taken to Kohala for the third time and inoculated by the usual methods on ten plants of Striped Tip cane, with ten plants punctured as controls with no inoculum. The results are shown in Table III:

TABLE III

Results of Inoculations Made With Reisolated Organism B and With Check Punctures With No Inoculum. Inoculations Were Made April 29, 1924, and Results Observed May 16, 1924.

Stalk Number	Organism	Positive results on old leaves		Positive results on young leaves	
		Number	Per cent	Number	Per cent
111	None	0	0	0	0
112	"	0	0	0	0
113	"	0	0	0	0
114	"	0	0	0	0
115	"	0	0	0	0
116	"	0	0	0	0
117	"	0	0	0	0
118	"	0	0	0	0
119	"	0	0	0	0
120	"	0	0	0	0
121	B	4	80	4	80
122	B	5	100	4	80
123	B	5	100	4	80
124	B	lost	0	lost	0
125	B	lost	0	lost	0
126	B	5	100	5	100
127	B	5	100	5	100
128	B	5	100	5	100
129	B	5	100	5	100
130	B	5	100	5	100

Organism B was reisolated from the positive results on several of the plants from 121 to 130.

The total number of inoculations may be summarized as follows: With Organism A, 14 leaves showed positive results from a total of 69 leaves, or 20.3 per cent; of the 345 individual punctures with Organism A, only 24 were positive, or 6.9 per cent. With Organism B, 47 leaves gave positive results from a total of 55 leaves, or 85.5 per cent, and of 175 individual punctures 113, or 64.5 per cent, formed definite red stripes. The inoculations with the controls and other organisms, namely C, D, E and X, all gave absolutely negative results.

All plants indicated as "lost" in the tables were not included in the foregoing summary. Plants 31 to 40 were not included in the number of positive cases of individual punctures, since observations were not made for the individual punctures on these leaves. Plants 106 to 110, since they were inoculated without punctures, were also left out, although the leaves inoculated showed 100 per cent infection.

The rules of proof have been followed and the results show definitely that the whitish organism, which for convenience we have called Organism B, is the cause of red-stripe disease of Tip canes at Kohala. Red stripe resulting from inoculation of Organism B is shown in the photograph reproduced in Fig. 1. This may be compared with naturally occurring red stripe as shown in Fig. 2.

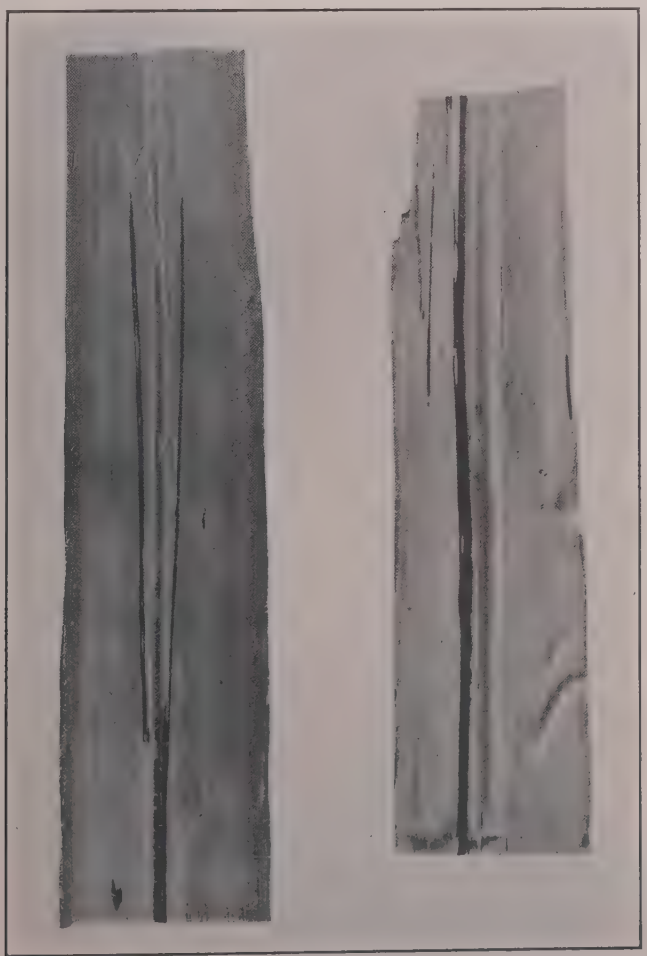

Fig. 1. Red-stripe disease produced artificially by inoculation with organism B from cultures.

Fig. 2. Natural infection with red-stripe disease of several leaves forming top of stalk of Yellow Tip variety.

Since completing these isolation and inoculation studies, inoculations have been made with Organism B in testing out the susceptibility of various parts of the cane plant, in determining resistance of tissues with age, and in testing out varieties for susceptibility or resistance; the results have shown that red-stripe disease can be reproduced at will with inoculations of Organism B on the leaves of the Tip varieties.

In the later stages of these inoculation and isolation studies, the morphologic and cultural characters of Organism B were being studied by Miss Helen A. Purdy; methods of diagnosis and easy determination of the organism were developed by Miss Purdy which have greatly assisted in obtaining the results presented in this paper. In locating fields for inoculation studies and in the actual work of the first series of inoculations, this project has been very greatly assisted by Mr. Jennings.

Description of the Organism Producing Bacterial Red-Stripe Disease of Sugar Cane

By Helen Alice Purdy[1]

In preceding publications on bacterial red-stripe disease of Tip canes, (4, 5),[2] a detailed account of the isolation and inoculation work has been given, showing the pathogenicity of a bacterial rod-shaped organism. From the numerous pure cultures of this organism isolated, three different cultures giving positive results on reinoculation were selected for a study of morphologic and cultural characteristics.

The methods of pure culture study, recommended by the Society of American Bacteriologists in the Manual for 1923 (1), have been followed closely. Unless otherwise stated, standard media have been used and the reaction adjusted before sterilization to neutrality with brom-thymol blue pH 7.0, by comparison with buffer solutions of known concentration. The final reaction here recorded, was determined after sterilization. The cultures used for inoculation of media were grown on 1 per cent glucose agar, incubated at 26° C. and transferred daily.

Morphologic Characters

Vegetative Cells: The organism is a short rod with rounded ends, occurring singly but more commonly in pairs. Occasional chains of no more than six rods were observed. On 1 per cent glucose beef-extract agar strokes, incubated at 26° C. for 48 hours and stained with gentian violet, the size of the majority is 1.67 μ by 0.7 μ.

With carbol fuchsin and gentian violet, smears from agar cultures show a more densely localized staining, bipolar in many cases. These more deeply staining areas are not alone confined to the poles, but frequently a single deeply stained area occurs in various parts of the rods. The appearance is to some extent distinctive of the organism.

Capsules, Sporangia and Spores: No capsules, sporangia or spores have been observed.

Motility: The organism from twenty-four-hour cultures in broth and on glucose agar shows marked motility. Flagella are readily stained from two day water cultures, incubated at 26° C., by Johnson and Mack's modified method (6). A single polar flagellum occurs most frequently, but occasionally two or three are attached to a pole; the bacterial rods with flagella are shown in Figs. 3 and 4.

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[1]This investigation was begun as a Bishop Museum Fellow of Yale University and completed, on expiration of the fellowship, as associate pathologist of the Experiment Station of the Hawaiian Sugar Planters' Association.

[2]Reference is made by number to "Literature cited," p. 17.

Figs. 3 and 4. Showing the flagella of the organism of red stripe of sugar cane X 1200. The photomicrographs are by H. Atherton Lee from a preparation by the writer.

Staining Reactions: The organism is easily stained with carbol fuchsin and gentian violet, but only faintly with methylene blue. Carbol fuchsin produces a definite bipolar staining from forty-eight-hour agar strokes. The organism probably must be considered Gram-negative; the organism *en masse* still exhibits a slight blue coloration following the washing with alcohol, but individual rods, although sometimes showing a faint blue color, were more frequently colorless. [3]

Cultural Characters

Agar Stroke: On 1 per cent glucose beef-extract agar pH 7.0, incubated at 27° C. for twenty-four hours, growth is moderate, filiform, flatly convex, shiny, smooth, opalescent, light buff (R.),[4] of rather slimy consistency, having a slight putrescent odor.

The light buff color is not so distinct on an agar medium, but it is plainly visible in a thick smear of the culture on a glass slide, held against a pure white background. The medium is unchanged in cultures of 5 or 6 days but in very old cultures is slightly browned in comparison with uninoculated media.

Agar Colonies: On 1 per cent glucose beef-extract agar pH 7.0, incubated at 26° C. for four days, surface growth is slow, circular, smooth, shiny, light buff (R.), with margin entire, flatly conic. In transmitted light, the colonies have a bluish cast. The range of size on dilution plates one week old is 1.75 mm. to 3.5 mm.; the size of the majority is 3 mm. The size of the colonies is dependent upon the number growing on one plate, the higher the dilution, the larger the colony becomes. Microscopically, the outer growth of the colonies appears finely granular with concentric markings, the center is grumose. In five to seven day cultures, the colonies are surrounded by a halo of cleared medium; colonies with such halos are shown in Fig. 5. The odor is slightly putrid. Submerged colonies are elliptic, ovoid, or punctiform, while deeply submerged ones, growing between the layer of medium and the bottom of the Petri dish, are effuse and grumose in structure.

Gelatin Stab: Stab cultures were made in 12 mm. tubes of non-nutrient gelatin (120 grams gelatin to 1000 cc. distilled water) with a reaction slightly acid, pH 6.7, and incubated at room temperature. Growth was scant, best at the top and slight and filiform at first along the line of puncture. Liquefaction was at first crateriform and very slow, but became stratiform and at the end of thirty days many of the tubes showed complete liquefaction with a heavy deposit of sediment in the bottoms of the tubes. Control tubes were

[3] The foregoing results were checked by Mr. Lee and Mr. Martin by comparison of the reaction of the red-stripe organism with that of *Lactobacillus* (*Bacillus*) *acidophilus*, which is strongly Gram-positive, and with *Salmonella* (*Bacillus*) *paratyphi* (*paratyphosus*) which is clearly Gram-negative. The red-stripe organism was found to be not so clearly Gram-negative as *S. paratyphi* but was far from the deeply stained color of *L. acidophilus* treated by the same method. The reaction was tried repeatedly on preparations from old and young cultures and with various gentian-violet preparations, always with the same result. All observers have agreed that it should be considered Gram-negative.

[4] R. refers to Ridgway, see (7) "Literature cited," p. 17.

unchanged. Similar results were obtained with stab cultures in nutrient gelatin in 12 mm. tubes, although the liquefaction was more rapid.

Gelatin Colonies: Seven-day growth on non-nutrient gelatin pH 6.7 was slow, circular, with heavier growth in the center, surrounded by scant growth, which was translucent and occurred in concentric rings. The slight saucer-shaped depressions of the colonies were the only evidences of liquefaction. The internal structure of the colonies was coarsely granular in the center and finely granular around the edge. The color of the heaviest growth was light buff (R.), becoming paler with each concentric ring of growth, until the outer ring was barely visible. The range of size was 1.5 mm. to 8 mm., the size of the majority, 3 mm.; colonies on nutrient gelatin pH 7.0 showed the same characteristics but very much heavier growth and a deeper buff color. The concentric markings were not so pronounced.

Nutrient Broth: Twenty-four-hour growth in beef-extract broth pH 7.0 incubated at 27° C. produces moderate clouding, no surface growth, no odor, scant sediment which becomes viscid on agitation, and with repeated shaking breaks up to form a part of the general cloudiness of the culture. Forty-eight-hour growth shows the formation of a pellicle, with the heaviest growth in the center, where it finally becomes weighted down in five-day cultures and hangs in strands which later break up into flaky particles, falling to the bottom of the tube. The amount of sediment increases with age until in a five-day culture it rises in a viscid column on agitation, which may persist for a minute before finally settling down again in the bottom. Violent shaking of old cultures produces turbidity.

Potato Cylinders: A forty-eight-hour culture, incubated at 26° C. shows moderate filiform growth, flatly convex, smooth, opaque, with a dull lustre on dry cylinders and shiny on moist potato. The potato was darkened. The color of the culture deepens with age, passing through shades of warm buff (R.) in forty-eight-hour cultures, tawny (R.) in five-day growth, to russet (R.) in six-day cultures. No odor is present. The consistency is butyrous. No diastasic action is evident with iodine on ten-day cultures, but with greater time cultured potatoes show a faintly perceptible port wine color indicating the presence of amylodextrin. Uncultured potatoes in comparison are the deep pure blue color showing no reduction of starch. The organism therefore exhibits a slowly diastasic action.

Chromogenesis: The organism is light buff (R.) on gelatin and beef-extract, glucose and nitrate agar, and warm-buff, tawny or russet (R.) on potato cylinders.

Oxygen Requirement: The organism is a facultative anaerobe. In fermentation tubes of beef-extract broth plus 1 per cent glucose, growth was present in the closed arm after ten days incubation at 27° C.; there was more abundant growth in the open arm, however.

Indol Production: No indol is produced in Frieber's trypsinized (1, p. B 31) broth pH 7.0, incubated at 26° C. for thirty days. The cultures were tested frequently by the Salkowski, vanillin and Ehrlich tests (1, p. B 31).

Dunham's peptone solution pH 7.0, also gives negative results, with fourteen-day cultures, incubated at room temperature, by the Salkowski and Ehrlich tests. With vanillin and concentrated sulphuric acid, both the inoculated and control tubes turn the same shade of orange. A strong putrescent odor is present.

Diastasic Action: Action upon starch is very slow and very slight and on some media entirely absent. In twenty-two days, streak plates of 0.2 per cent soluble starch in beef-extract agar pH 6.7 show profuse growth but the tests for diastasic action were inconclusive. Cultures of peptone solution containing 1 per cent corn starch pH 7.0 show a port-wine color with a 3 per cent iodine solution after 20 days, indicating the presence of amylodextrin and other lower products; uninoculated tubes of the same medium give the deep purple color of starch with iodine. A similar very slight diastasic action was evident with cultures on potato cylinders after 20 days. The diastasic action of this organism, however, is so slight as to require careful observation to be detected.

Hydrogen-Sulphide Production: Sixteen-day-old stab cultures in lead-acetate beef-extract agar pH 6.8 showed no perceptible browning of the medium along the line of puncture.

Lead-acetate paper, inserted in the cotton plugs of cultures incubated at 26° C. remained negative after twenty days over nutrient broth, nutrient gelatin, Dunham's peptone solution, glucose nutrient-agar strokes and potato cylinders.

Milk: No coagulation takes place. Clearing begins in one week and is completed in forty days. The reaction of the milk, in high dilution, was tested in 1, 2, 4 and finally in 37 days in the inoculated tubes. There was no noticeable alteration in the reaction, using brom-thymol blue and brom-cresol purple as indicators. A browning of the milk occurs and the odor of protein decomposition is very strong.

Reduction of litmus began in three days and was completed on the eighth day.[5] Reduction of methylene blue (0.75 cc. of a 10 per cent aqueous solution to 200 cc. of milk) was very rapid and took place in forty-eight hours.

Nitrate Reduction: On 1 per cent potassium-nitrate beef-extract agar pH 7.1, incubated at 26° C. nitrates were promptly reduced. Preliminary tests for nitrites on 24 and 43-hour cultures were made with sulphanilic acid and alpha naphthylamine (1, p. B 27) giving strong positive results. With these reagents, a trace of pink appeared on one-hour cultures five minutes after applying the test solutions, while a deep pink appeared at once on six-hour cultures. No gas was formed in nitrate-agar shake cultures.

Ammonia Production: Ammonia was produced in three-day-old cultures in Dunham's peptone solution, four-day-old cultures in Frieber's trypsinized broth, and forty-eight-hour cultures on one-tenth per cent potassium-nitrate agar. Positive tests were obtained with both Nessler's solution and moist litmus paper. In the case of the former, the characteristic orange color was produced when adding the reagent to the cultures, indicating the presence of ammonia. With the latter test, moist litmus paper was inserted in the cotton plug and the contents of the tube were gently heated. In the inoculated tubes

[5] In this test 6 cc. of a 1 per cent solution of azolitmin was used with 200 cc. of milk.

the paper turned blue as the ammonia, driven off from the cultures, reached the moist paper, but in the control tubes the paper remained pink.

Fermentation Tests: Cultures in beef-extract broth plus 1 per cent glucose pH 6.8, lactose pH 7.0, sucrose pH 7.3, and glycerine pH 7.0, incubated for ten days at 27° C., produced no gas.

Series of cultures were prepared in a synthetic medium of potassium chloride, 0.2 gram; dibasic ammonium hypophosphate, 1 gram; distilled water, 1000 cc. plus 1 per cent glucose, sucrose, lactose, arabinose, xylose, mannit, levulose and raffinose respectively. These were incubated for eight days at room temperature and formed no gas, although growth occurred in every case.

Indicator Sugar Agar: Equal parts 0.04 per cent solutions of brom-cresol purple and cresol red (6 cc. of the mixture of indicators to 160 cc. of medium) were added to beef-extract agar pH 7.0 with 1 per cent each of glucose, lactose, sucrose and glycerine. Stroke cultures, incubated at 26° C. gave an alkaline reaction in two days with all the carbohydrates, but not so pronounced in the case of glucose. At the end of five days, the tubes of glucose agar showed varying amounts of acid present while the reaction in the lactose, sucrose and glycerine tubes was strongly alkaline. In fifteen days some of the glucose-agar cultures showed a neutral or slightly alkaline reaction, while others of the glucose-agar cultures still gave an acid reaction. The other sugar cultures all remained strongly alkaline. The amount of acid present gradually decreased with time after the fifth day in the glucose-agar cultures.

Synthetic Peptone-Free Sugar Agar: The same amounts of sugars and mixed indicator solutions, used above, were added to a synthetic agar of potassium chloride, 0.2 gram; dibasic ammonium acid phosphate, 1 gram; agar, 15 grams; distilled water, 1000 cc. Stroke cultures, incubated at 26° C. gave practically no growth with sucrose, lactose, and glycerine, while growth was moderate with glucose. In two days, an acid reaction was evident in all of the inoculated tubes of glucose agar, and in four days, the acid reaction was complete throughout the inoculated glucose medium. No gas was produced with any of the sugars.

Litmus Sugar Agar: On stroke cultures of beef-extract litmus agar pH 7.0 containing 1 per cent glucose, sucrose and glycerine, incubated at 26° C., growth was profuse, but no acid or alkaline reaction was evident in thirty days. With glucose, reduction of litmus began in four days and was complete in 15 days. Reduction of litmus was much slower in glycerine agar but was nevertheless definite. Reduction did not take place in the sucrose agar; the cultured tubes remained the same color as the uncultured tubes of the medium.

Effect of Physical Conditions

Thermal Death Point: Test tubes of uniform size, containing 10 cc. beef-extract broth were inoculated with two loops full of a fifteen-hour broth culture and immersed in a water-bath to the neck of the tube for ten minutes at temperatures varying from 40° to 60° C. A standardized thermometer was used and temperature readings taken with a hand lens at two-minute intervals during

the exposures. The fall in temperature never exceeded one-eighth of a degree during a ten-minute exposure. The resistance of the organism to heat is lowered in the presence of alkali. In preliminary experiments, the thermal death point was found to lie between 50° and 51° C. in alkaline broth and between 52° and 53° C. in neutral broth. Tests finally placed the thermal death point at 51° C. in alkaline broth pH 8.6, and 52° C. in neutral broth pH 7.0 as shown in Table I.

TABLE I

Showing the Results of Exposures of the Organism of Red Stripe of Sugar Cane for Ten-Minute Periods at Various Temperatures

	Broth pH 8.6			Broth pH 7.0				
No. of tubes	4	4	2	2	4	4	4	2
Temperature	50° C.	51° C.	52° C.	51° C.	52° C.	53° C.	54° C.	55° C.
Growth in 4 days.....	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent

This experiment was repeated with identical results.

Desiccation: Preliminary experiments with infusions of cultures scraped from agar strokes proved variable and unsatisfactory for uniform results as to the resistance of the organism to drying under a given set of conditions. With this method, 45 cover glasses of a total of 47, with smears of the organism, placed in nutrient broth after five days drying, gave no growth. After six days of drying 1 cover glass showed growth from a total of 23 cover glasses. After eight days of drying, 2 cover glasses from a total of 68 produced growth in nutrient broth. The following method gave more clear-cut results: one loopful of an eighteen-hour nutrient-broth pH 7.0 culture, incubated at 26° C. was placed in the center of a sterile cover slip, contained in a sterile Petri dish and allowed to dry in a dark closet. At intervals of 2, 3, 6, 8, 9 and 24 hours, such cover slips were dropped into sterile nutrient broth and incubated at 26° C. for six days. No growth occurred in any of the six tubes where the culture had dried 24 hours. Results showed the period of resistance to desiccation, under the conditions of this experiment, to fall considerably within 24 hours.

Exposure to Sunlight: Series of three agar dilution plates each were exposed to direct sunlight on cracked ice, between twelve and one o'clock (Honolulu, T. H., May 14, 18, 23, 1924) and submitted to 5, 10, 15, 20, 25 and 30-minute exposures. Half of each plate was shaded with black paper and covered with white paper. Five days incubation at 26° C. showed that all the organisms in the highest dilution plates and a very large percentage of those in the lowest dilution were killed by 15-minute exposures. At 20-minute exposures, 100 per cent of the organisms in the three different dilution plates were killed. These results are shown in the photographs, Figs. 5 and 6.

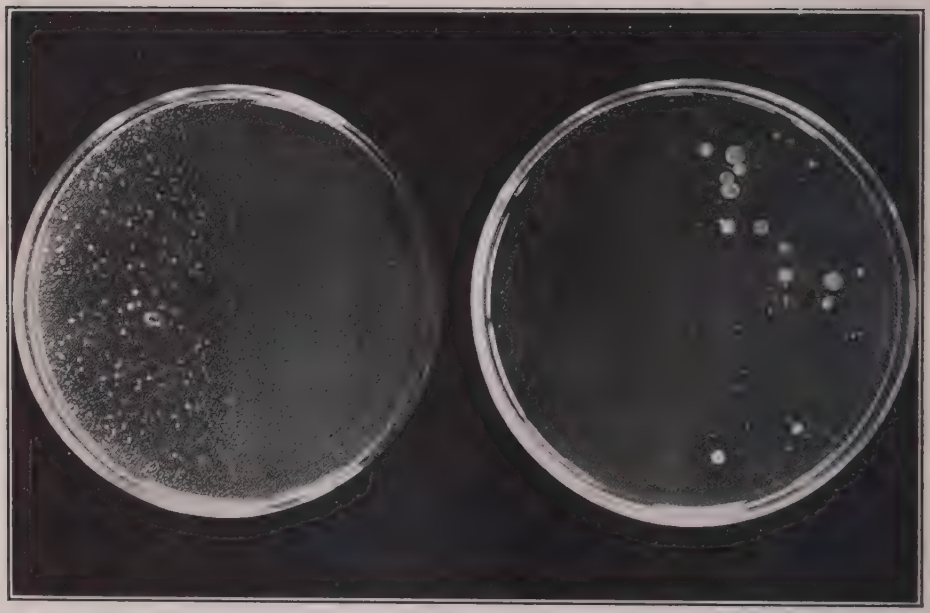

Fig. 5. The second and third of a series of three dilution plates, one half of each having been exposed to direct sunlight for fifteen minutes. The grumose character of the centers of surface colonies on agar is also shown, as well as the ovoid to elliptic shape of buried colonies. Photograph by H. Atherton Lee.

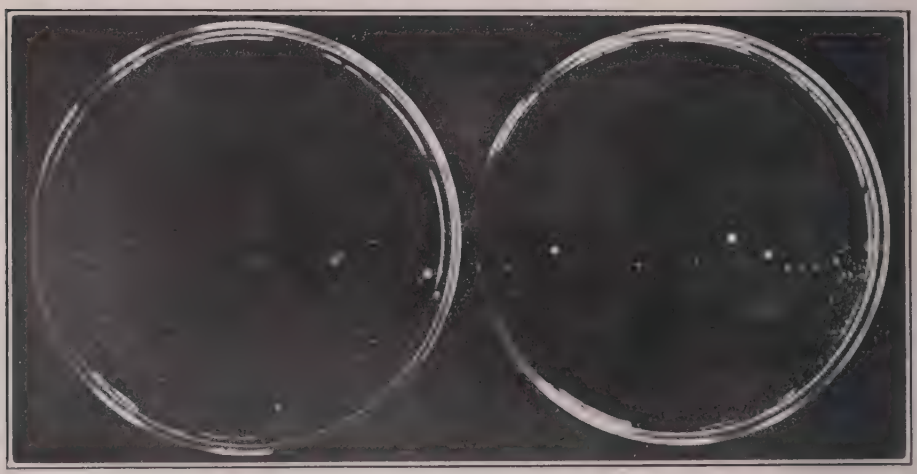

Fig. 6. The second dilutions of two series of poured plates, one half of each having been exposed to direct sunlight for twenty minutes. Under the conditions of the experiment all individuals of the organism were killed in such twenty-minute exposures. Photograph by J. P. Martin.

Systematic Position of the Organism

Identification: According to the descriptive chart of the Society of American Bacteriologists for 1920 (1), the index number of the organism is 5322-32120-1233.

The organism agrees in a great many cultural characters with Miss Elliott's (3) species on proso millet, *Bacterium panici*, and because of the similarity of the lesions caused by *B. panici* with those on sugar cane, the sugar cane organism should be tentatively considered similar until cross inoculations are possible.

The failure of the proso organism to produce an acid reaction with glucose, a characteristic of the cane organism, may be due to the fact that the production of acid was too slight to be detected in the presence of the buffered peptone medium on which it was grown, or the acidity produced may have been obscured by the alkalinity produced from the peptone (1, p. A 24). The explanation probably lies in the latter statement since cultures of the cane organism in plain nutrient broth and Dunham's peptone solution show a marked alkaline reaction when compared with the neutral controls. The true behavior of the cane organism toward carbohydrates other than glucose can obviously not be determined until growth is obtained on indicator medium that is peptone free. The cane organism, in common with the proso organism, produces no detectable acid in the case of glucose with litmus as an indicator. The discrepancy in the results of the desiccation experiments with the proso and cane organism may be due to different methods of investigation.

This investigation was carried out in the spring of 1924. To H. P. Agee, director of the Experiment Station of the Hawaiian Sugar Planters' Association, I wish to express my appreciation of the courtesies of the laboratory extended to me as a Bishop Museum Fellow. To H. Atherton Lee, head of the Department of Pathology and his associate, J. P. Martin, I am most grateful for valuable suggestions and the opportunity of describing the organism of their isolation studies. H. A. Cook, of the Department of Sugar Technology, gave valuable assistance in the preparation and loan of indicator, buffer and test solutions. I should like to acknowledge the kindly cooperation of all the members of the Sugar Technology and Pathology Departments.

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Diagnostic Studies of the Organism of Red-Stripe Disease

By H. Atherton Lee, J. P. Martin, and Helen A. Purdy[1]

This work was undertaken for the purpose of obtaining quick methods of identifying in culture, the bacterial organism causing red-stripe disease of Tip canes. It should be mentioned first, that throughout this paper where statements refer to pH values, they have been determined colorimetrically. The sugars used throughout the tests were prepared by the Special Chemicals Company of Highland Park, Illinois.

Determination of pH Values for Optimum Growth

The optimum hydrogen-ion concentration for growth was first determined in Dunham's peptone solution containing 2 per cent glucose. These optimum-growth tests were made in fermentation tubes in order that anaerobism and gas formation could also be observed. Eight series, with 6 fermentation tubes to each series, were prepared with pH values of 5.0, 5.6, 6.0, 6.6, 7.0, 7.6, 8.0 and 8.6. Five tubes in each series were inoculated and the sixth tube was held as a control. All inoculations were made with the same 3 mm. platinum loop; the inoculum was taken from a 3-day-old culture of the organism growing in a 2 per cent glucose Dunham's peptone solution, pH 7.0. The tubes were held at laboratory temperatures varying from 26° to 29° C.

At the expiration of 14 days the development and type of growth were observed; the results are shown in Table I.

Table I

Showing Character of Growth of Red-Stripe Organism at Expiration of 7 Days in 2 Per Cent Glucose Dunham's Peptone Solution at Various Hydrogen-ion Concentrations

pH Value	Ring formation	Pellicle	Clouding	Sediment	Odor	Growth in closed arm	pH determination after test	
							Controls	Cultures
5.0	None	None	None	None	None	None	5.4	5.2
5.6	None	Thin, flocculent	Thin, turbid	None	Slightly putrescent	None	6.0	5.6
6.0	None	Thin, flocculent	Moderate, uniform	None	Slightly putrescent	None	6.0	5.6
6.6	Present	Thin, uniform	Moderate, uniform	Slight, viscid	Slightly putrescent	None	6.3	6.2
7.0	Present	**Moderate, uniform**	**Moderate, turbid**	Slight, viscid	Slightly putrescent	None	6.6	6.2
7.6	Present	Thin, flocculent	Moderate, turbid	Slight, viscid	Slightly putrescent	None	6.6	6.5
8.0	Slight	None	Moderate, turbid	Slight, viscid	Putrescent	None	6.8	6.8
8.6	Slight	None	Thin, uniform	Slight, viscid	Putrescent	None	7.3	7.2

[1] The writers wish to express appreciation to Dr. C. S. Hudson of the Bureau of Standards, U. S. Department of Commerce, for many suggestions in the carrying out of this work and in the conclusions presented here.

The alteration of the hydrogen-ion concentration following autoclaving in the various series, is shown by the pH determinations made for the control tubes at the end of the experiment. In making statements concerning optimum growth and limits of growth these final determinations of the controls are, of course, the most essential. The red-stripe organism produced no growth in solutions of pH 5.4, and pH 6.0 is apparently close to the limit of hydrogen-ion concentration tolerated. The alkaline limit for growth was beyond the range of the series prepared, however. The tubes in the series with pH 7.3 apparently approached the alkaline limits for growth. Optimum growth occurred in the series prepared to pH 7.0 which gave a pH of 6.6 after autoclaving. Apparently the range of growth of this organism coincides in a general way with that of most bacterial plant pathogenes as determined by Quirk and Fawcett (1). In all series of the present tests, in glucose peptone broth, the pH values were lowered by the growth of the red-stripe organism.

The odor of cultures of the red-stripe organism is worthy of note. The slightly putrescent odor on the usual culture media is somewhat characteristic, and with experience the presence of a different odor enables one in isolation attempts, to throw out a number of similarly appearing white organisms.

Growth With Various Sugars

The growth in the different sugars was first undertaken in Uschinsky's[a] solution corrected to pH 7.0. The sugars to be tested were used at a concentration of 2 per cent. The Uschinsky's solution without the sugars was first autoclaved, the proper sugar added to each series, and with a few exceptions such series were steamed for 15 minutes each day for 3 successive days. In the case of levulose, raffinose and sucrose, the media, after the addition of the sugars, were passed through autoclaved Chamberland filters into previously sterilized fermentation tubes. After filtration these media were held for 3 days to observe the presence of any contamination which might have taken place during the filtration; no contamination occurred. Each series consisted of six fermentation tubes of which five were inoculated and one was held as a control.

All cultured tubes of all series were inoculated with the same 3 mm. loop from a 3-day-old culture of the red-stripe organism in a 2 per cent glucose Uschinsky's solution and held at laboratory temperatures. The development of growth is shown in Table II.

The most notable feature of this series of tests was the entire absence of gas formation from any of the sugars tested. In discussing the results shown in Table II, it is interesting that in the media of Uschinsky's solution to which the pentoses, arabinose and xylose, and the pentose alcohol, arabitol were added, growth was almost entirely absent. Growth occurred readily in the same medium to which the hexoses were added: growth was more abundant in glucose, galactose and levulose than in mannose. Growth in the hexoses was apparently

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[a] The Uschinsky's solution as used here contained:

Sodium asparaginate	—	4 grams
Sodium chloride	—	5 "
Calcium chloride	—	0.1 "
Magnesium sulphate	—	0.3 "
Dipotassium phosphate	—	2.5 "
Ammonium lactate	—	6.0 cc.
Distilled water	—	1000.0 cc

Table II

Showing the Character of Growth of the Red-Stripe Organism at the Expiration of 28 Days in Series of Uschinsky's Solutions to Which 2 Per Cent of Various Sugars Had Been Added

Sugar	Ring formation	Growth in open arm: Pellicle	Clouding	Sediment	pH Value: Control	pH Value: Cultures	Growth in closed arm
Arabinose	None	None	None	None	6.0	6.0	None[1]
Xylose	None	None	None	None	6.0	6.0	None
Levulose (filtered)	**Heavy**	**Floculent**	**Turbid**	**Viscid**	6.4	7.6	**Present**[2]
Galactose	**Moderate**	**Thin, flocculent**	**Turbid**	**Slight, viscid**	6.0	8.2	**Moderate, uniform**[3]
Mannose	None	Thin	Moderate, uniform	Slight, viscid	6.0	8.4	None
Glucose	**Moderate**	**Thin, flocculent**	**Turbid**	**Slight, viscid**	6.0	8.0	**Faint, uniform**[4]
Sucrose (filtered)	Present	**Flocculent**	Turbid	Viscid	6.0	8.0	None
Sucrose (steamed)	Dense	Flocculent	Turbid	Viscid	6.0	8.4	None
Maltose	Moderate	Thin, flocculent	Turbid	Viscid	6.2	8.4	None
Trehalose	None	Thin	Heavy, turbid	Heavy, viscid	6.0	8.2	None
Lactose	None	None	Turbid	Moderate, viscid	6.0	8.0	Faint, uniform[5]
Raffinose (filtered)	Slight	Flocculent	Turbid	Viscid	6.2	8.0	Faint, uniform[6]
Dulcitol	Slight	Thin, flocculent	Turbid	Slight, viscid	6.0	8.2	Faint, uniform[3]
Sorbitol	Slight	Thin, flocculent	Turbid	Slight, viscid	6.0	8.2	Faint, uniform
Arabitol	None	None	None	None	6.0	6.0	None

[1] One tube in the arabinose series showed a light ring, thin flocculent pellicle, heavy uniform clouding, and slightly viscid sediment in the open arm; in the closed arm a moderate uniform clouding. When tested out to prove the identity of the organism causing clouding it was shown to be the red-stripe organism.

[2] One tube in the levulose series showed growth in the closed arm; it was plated out and found to be a pure culture of the red-stripe organism.

[3] Two tubes of the five in these series did not show growth in the closed arm.

[4] Growth occurred in the closed arms of all glucose tubes.

[5] Growth was present in the closed arms of only 2 of the 5 cultures. Cultures were plated out and found to be uncontaminated.

[6] One of the five cultures showed faint uniform growth in the closed arm. It was plated out and found to be uncontaminated.

better than in the disaccharides. Growth in the sucrose medium sterilized by intermittent steaming in the Arnold sterilizer was better than in the filtered sucrose medium, indicating apparently that the steaming resulted in some degree of hydrolysis of the sucrose. Of the disaccharides, growth was better in sucrose, trehalose and maltose than in the lactose. Growth occurred in the trisaccharide, raffinose, but not as vigorously as in the majority of disaccharides, although better than in the case of lactose. Sorbitol and dulcitol, the hexose alcohols, supported growth but apparently with considerably greater difficulty than the hexoses.

Growth in the closed arm of the tubes occurred in one tube of the arabinose medium, one tube of the levulose medium, two tubes of galactose, all glucose tubes, two of the lactose tubes, one raffinose tube, two dulcitol tubes and two sorbitol tubes. It seems reasonable to conclude that the organism is a facultative anaerobe, but the conditions conducive to anaerobic growth are not clear.

The greatly increased pH value of the cultures, in every instance, as compared to the controls was notable; this feature was fairly uniform in all series of sugars in Uschinsky's medium in which growth occurred.

The experiment was repeated, using as a medium, Dunham's peptone solution pH 7.0 instead of Uschinsky's solution. In this case, in addition to the sucrose and raffinose, lactose, maltose, trehalose and starch media were prepared by passing through autoclaved Chamberland filters rather than by sterilization in the Arnold. After being filtered, these six media were held in the fermentation tubes for three days before inoculation, to observe the presence or absence of contamination. The other sugar media were sterilized by 15 minutes steaming on 3 successive days.

The development and character of growth is shown in Table III.

TABLE III

Showing the Character of Growth of the Red-Stripe Organism at the Expiration of 23 Days in Series of Dunham's Peptone Solutions to Which 2 Per Cent of Various Sugars Had Been Added

Sugars	Growth in open arm				Growth in closed arm	pH Value	
	Ring formation	Pellicle	Clouding	Sediment		Controls	Cultures
Arabinose	Slight	None	Slight, turbid	Slight	None	5.8	4.0
Xylose	Slight	Thin, flocculent	Moderate, uniform	Slight	None	...	...
Mannose	None	None	Moderate, uniform	None	None	6.8	6.4
Galactose	Slight	Thin	Moderate, uniform	Slight	None	6.8	5.4
Levulose	None	None	Moderate, uniform	None	None	6.4	5.0
Glucose	Slight	None	Moderate, uniform	Slight	None	6.8	5.2
Sucrose (filtered)	None	None	**Heavy, uniform**	**Heavy**	None	7.0	7.8
Trehalose (filtered)	None	None	Moderate, uniform	Slight	None	7.0	7.6
Maltose (filtered)	None	None	**Heavy, uniform**	**Heavy**	None	7.0	7.6
Lactose (filtered)	None	None	Moderate, uniform	Heavy	None	...	7.8
Raffinose (filtered)	None	None	Moderate, uniform	None	None	7.0	8.0
Starch (filtered)[2]	None	None	Slight, uniform	None	None	...	7.8
Glycerol	None	None	Slight, uniform	None	None	7.0	7.8
Arabitol	Slight	None	Moderate, uniform	Sught	None	7.0	7.8
Dulcitol	None	None	Moderate, uniform	Slight	None	7.0	7.8
Sorbitol	None	None	Moderate, uniform	Slight	None	7.0	7.6

[2] Two days after passing the starch medium through the Chamberland filters, five of the six fermentation tubes in which the medium was held showed contamination. Apparently the presence of filter passers in the starch created these contaminations. The observations recorded in the table above were made upon the one uncontaminated tube of the starch medium. None of the other filtered media showed contamination.

The outstanding phenomenon shown in Table III was the decrease of pH values by the organism in peptone media containing pentoses and hexoses, while the pH values were increased in the series containing disaccharides, and polysaccharides.

The absence of growth in the closed arms of fermentation tubes in this media as compared with the Uschinsky-solution sugar media is noteworthy. It is interesting also, that whereas the Uschinsky solution gave the best growth with the addition of the hexoses, in the peptone media the disaccharides gave the best growth.

Discussion of Results

Considering the series of sugars in Uschinsky's medium, it is apparent first that the pentoses and pentose alcohols will not support growth by this organism. A second notable feature, is the ready utilization of the hexoses in the metabolism of this organism. Thirdly, this organism is capable of hydrolyzing the disaccharides and utilizing the resulting hexoses to some extent; the hydrolysis of the trisaccharide raffinose was apparently more difficult, however, for growth was not as abundant. The strong increase in pH values throughout all series where growth occurred, probably arises from a utilization of the lactate and asparaginate molecules in the metabolism of the organism in excess of the utilization of the ammonium and sodium ions.

Considering next the results obtained with the sugars in Dunham's peptone solution, it is apparent that growth in the series of pentose media resulted from the peptone rather than the pentoses. This was tested out by placing the organism in Dunham's peptone solution with the absence of all sugars; growth resulted readily. Of greater interest was the strong increase in hydrogen-ion concentration in the series containing monosaccharides; it is probable that there is a considerable oxidizing capacity of the red-stripe organism in changing these sugars to carbohydrate acids. It seems evident that the organism hydrolyzes peptones to form amino acids and liberates presumably ammonium ions which increase the pH value of the peptone media. This production of alkaline ions is apparently in excess of the formation of carbohydrate acids in the case of the disaccharide and trisaccharide media. The more ready metabolism of this organism on the hexoses, on the other hand, results in considerably stronger formation of carbohydrate acids than results from the disaccharides, trisaccharides and polysaccharides, and this acid formation is in excess of the liberation of alkaline ions from the hydrolysis of the peptone. In the series of sugars in the peptone solutions, the more abundant growth from the disaccharides than from the hexoses, is explained by formation of carbohydrate acids in the case of the hexoses and the increase in hydrogen-ion concentration beyond the limits of growth for the organism.

A diagnostic test in culture becomes apparent from these results, which depends upon the oxidizing effect of the organism on hexoses to form acids, its poor hydrolyzing effect upon disaccharides and its ready hydrolysis of peptone, forming alkaline-reacting products. This combination of characters can be determined upon two series of peptone media, one containing a hexose such as glucose, and the second a disaccharide such as sucrose. The excess of hydrogen-ion forma-

tion in the glucose media and the excess of alkaline-ion formation in the sucrose media, both easily shown by the addition of indicators to the cultures, will indicate an organism very similar to the red-stripe bacteria. Such a result was indeed obtained by Miss Purdy (2) in her cultural studies in the instance where beef-extract indicator agar with glucose gave an acid reaction, and with glycerin, sucrose and lactose gave an alkaline reaction.

Peptone beef-extract agar slants pH 7.0 containing 2 per cent of arabinose, xylose, levulose, galactose, sucrose, maltose, raffinose, arabitol and dulcitol were prepared; to these series of sugar media an indicator containing equal parts of aqueous .04 per cent brom-cresol purple and .04 per cent cresol red was added at the rate of 1 part to 25 parts of the media. The organism, in the presence of these indicators, on the hexose agars turns the medium a brilliant yellow, indicating hydrogen-ion increases; and on the pentoses and the disaccharides and the trisaccharide tested, the agar is turned a brilliant purple even more rapidly, indicating an excess of alkaline ions. The acid reaction is shown much more rapidly in the case of glucose and galactose than in the case of levulose.

The same reaction is obtained on Uschinsky's agar with the same indicators added, with galactose and glucose, but much more slowly. Apparently the hydrolysis of the asparaginate molecule in the Uschinsky's solution gives a greater excess of alkaline ions than the hydrolysis of the peptone in the Dunham's solution. The balance between the formation of acid ions and alkaline ions is apparently fine in the glucose and sucrose peptone agars, thus giving the quickest diagnostic test in such media.

Growth in the Absence of Oxygen

Following the tests for growth in peptone media in fermentation tubes, in which no growth occurred in the closed arms of the fermentation tubes in the case of any sugar, it seemed well to test the anaerobism of the red-stripe organism by other means.

Tests to grow the organism in a partial vacuum for which the manometer showed a height of 28 inches of mercury, were unsuccessful due to the drying out of the medium where such a vacuum was maintained.

Poured plates of the organism in 2 per cent glucose agar pH 7.0 were then placed in Novy jars and the air exhausted until the manometer showed a height of 28 inches of mercury. This vacuum was then released by running in carbon dioxide until the concentration was again equivalent to atmospheric pressure. The vacuum pump was again applied until a manometer reading of 28 inches was secured and carbon dioxide again supplied to replace the vacuum. Poured plates under the atmospheric conditions of the laboratory were maintained as controls.

Growth appeared in the Petri dishes in the Novy jars somewhat slowly as compared with the control plates. At the end of ten days the plates were removed from the Novy jars; abundant colonies of the red-stripe organism occurred in the plates, identical to those of the control plates except that they were slightly smaller. The absence of oxygen at the termination of the experiment was shown by the extinguishing of a flame in the experimental Novy jar, while in a control Novy jar the same flame burned without difficulty.

The experiment was repeated, substituting hydrogen for carbon dioxide. Growth took place similarly in the presence of hydrogen and absence of oxygen, as in the previous test where carbon dioxide was used to displace the oxygen. In this case also the colonies were somewhat smaller than those formed under laboratory atmospheric conditions, but otherwise similar in every way.

Apparently the conclusion is warranted that the red-stripe organism is a facultative anaerobe. The absence of anaerobic growth in the peptone media in fermentation tubes is a matter of conjecture entirely.

Summary

(1) The optimum hydrogen-ion concentration for growth of the red-stripe organism in culture is between pH 6.6 and pH 7.0. Growth of the organism is inhibited in media of pH 5.4; the concentration of alkaline ions inhibiting growth is at values higher than pH 7.3.

(2) Growth in Uschinsky's solution is best in the presence of hexoses, but some hydrolysis of disaccharides and trisaccharides takes place which will support the metabolism of the organism. The pentoses tested will not support growth in Uschinsky's solution.

(3) The utilization of the lactate and asparaginate molecules in the metabolism of this organism results in an excess of alkalinity when growth takes place in Uschinsky's solution containing sugars. In Dunham's peptone solution also the hydrolysis of the peptone results in the liberation of alkaline ions.

(4) The oxidation of the monosaccharides in peptone media results in the formation of carbohydrate acids and gives an excess of hydrogen ions over the alkaline ions formed from the hydrolysis of the peptone. In peptone media containing disaccharides and trisaccharides the hydrolysis of such sugars is not sufficient to enable acid formation to take place as in the case of the monosaccharides. There results, therefore, an excess of alkalinity in peptone media containing disaccharides and trisaccharides.

(5) A diagnostic test is possible by comparing growth on monosaccharides and disaccharides in peptone beef-extract agar containing cresol red and bromcresol purple. In the case of the monosaccharides, strong acid formation turns the medium an intense yellow, and in the case of the disaccharides the medium is turned an intense purple. For this test glucose as a monosaccharide and sucrose as a disaccharide give the quickest and best results.

(6) The organism in glucose-agar poured plates in Novy jars, in the presence of carbon dioxide and absence of oxygen makes growth with no great difficulty. Similar results were obtained in the presence of hydrogen. The organism is apparently a facultative anaerobe.

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The Effect of Disinfectants on the Organism Causing Red Stripe of Sugar Cane

By J. P. Martin

Although it does not seem probable that the use of disinfectants will be feasible in controlling red-stripe disease of sugar cane in the field, the knowledge of the action of disinfectants is desirable for preventing the spread of the disease into unaffected districts and in quarantine or, if necessary, eradication methods. For this reason, a study was undertaken of the action of disinfectants upon the bacterial organism causing red-stripe disease of sugar cane.

Anderson and McClintic[1] have devised methods for determining the phenol coefficient of disinfectants; Lee[2], in citrus-canker studies, adapted these methods for the determination of the toxic action of fungicides and disinfectants against bacterial organisms. Similar methods were adopted in the studies reported in this paper.

These methods were, in brief, as follows: All tests were made without the presence of organic matter. Five cubic centimeters of the various dilutions of the disinfectants to be tested were placed in uniform vials, 1 inch in diameter by 3 inches in length. These small vials containing the different concentrations were maintained at 20° C. for the duration of the test, by submersion in a carefully controlled water bath. In order that the organism to be tested would be uniform throughout the experiments, three-day-old cultures in beef-extract peptone bouillon pH 7 were kept available for each series of tests. To each dilution of the disinfectants, 0.1 cubic centimeter of such a three-day-old culture was added by means of sterile, graduated pipettes and agitated in order to distribute the organism evenly in the disinfectant. After exposures of 2.5, 5, 7.5, 10, 12.5 and 15 minutes at each concentration, one 3 mm. loopful of each of the various inoculated disinfectants was transferred to separate sterile beef-bouillon, pH 7, tubes. Each platinum loop was carefully flamed before making the transfer. Extreme care was taken to secure the inoculated disinfectant from the center of the vial in order not to transfer any organism that had not been in direct contact with the disinfectant. The inoculated bouillon tubes were incubated for five days at room temperatures, which usually varied from 25 to 28° C. At the end of this period, these tubes were examined and recorded for growth, or absence of growth, as indicated by cloudiness or clarity of the bouillon. In doubtful or suspicious cases, diagnostic tests were made in order to preclude any possibility of recording a contamination as a survival of the red-stripe bacteria. Since this

[1] Anderson, John F., and McClintic, Thomas B., Method of Standardizing Disinfectants With and Without Organic Matter, Bull. Hyg. Lab. 82 (1912).

[2] Lee, H. Atherton, Action of Some Fungicides on the Citrus Canker Organism. The Philippine Journal of Science, Vol. 17, No. 4, October, 1920.

organism reduces nitrates very rapidly, as shown by Miss Purdy,[3] streak cultures were made on nitrate-agar slants and after twenty-four hours were tested for the presence of nitrites.

The results of each disinfectant used are tabulated in the following tables, growth being represented by the + sign and absence of growth by the — sign.

TABLE I

Results of Exposures of the Red-Stripe Organism in Dilutions of Phenol. Tests Made June 4, 1924, and Observed June 9, 1924

Length of exposure	Dilutions in terms of per cent									
Minutes	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
2.5	+	+[a]	—	—	—	—	—	—	—	—
5.0	+	—	—	—	—	—	—	—	—	—
7.5	+	—	—	—	—	—	—	—	—	—
10.0	+	—	—	—	—	—	—	—	—	—
12.5	+	—	—	—	—	—	—	—	—	—
15.0	+	—	—	—	—	—	—	—	—	—

In addition to the results shown in Table I, a duplicate set of determinations was made with phenol and the results obtained were identical with those tabulated above. A 1 per cent phenol solution at exposures of 5 minutes or longer readily kills the organism, while a dilution of 0.5 per cent gave positive results at all exposures. If this disinfectant is to be employed, it would not be safe to use less than a 2 per cent solution of phenol for entire disinfection. Lee has shown that a dilution of 1 to 100, or a 1 per cent solution of phenol would be sufficient to kill the citrus-canker organism, *Pseudomonas citri,* Hasse, in the absence of organic matter. From these results it is apparent that the red-stripe organism requires a 1 per cent stronger solution of phenol than *P. citri.*

TABLE II

Results of Exposures of the Red-Stripe Organism in Dilutions of Mercuric Bichloride. Tests Made June 4, 1924, and Observed June 9, 1924.

Length of exposure	Dilutions									
Minutes	1 to 1,000,000	1 to 500,000	1 to 200,000	1 to 100,000	1 to 50,000	1 to 20,000	1 to 10,000	1 to 5,000	1 to 2,000	1 to 1,000
2.5	+	+	+	+	+	—	—	—	—	—
5.0	+	+	+	+	+	—	—	—	—	—
7.5	+	+	+	+	+[a]	—	—	—	—	—
10.0	+	+	+	+	—	—	—	—	—	—
12.5	+	+	+	+	—	—	—	—	—	—
15.0	+	+	+	+	—	—	—	—	—	—

(a) Tube tested for red-stripe bacteria on nitrate agar, June 9, 1924; positive June 10, 1924.

[3] Purdy, Helen A., Description of the Organism Producing Bacterial Red-Stripe Disease of Sugar Cane. This bulletin, page 9.

A slight difference was noted in the two tests made with mercuric bichloride, and the results as shown in Table II are those of the first test. In the second test, growth took place in dilutions of 1 to 50,000, at exposures of 2.5 and 5 minutes, in 1 to 100,000 at exposures of 2.5, 5, 7.5 and 10 minutes, and in 1 to 200,000 at all exposures. A dilution of 1 to 20,000 or even 1 to 10,000 is the weakest solution that could be recommended with any degree of safety against this organism. Mercuric bichloride 1 to 1,000 is a very common disinfectant used in laboratories for general disinfection, and it is readily seen from the above results that such a solution is sufficiently toxic to insure entire disinfection.

Table III

Results of Exposures of the Red-Stripe Organism in Dilutions of Lysol. Tests Made June 5, 1924, and Observed June 10, 1924.

Length of exposure	Dilutions									
Minutes	1 to 1,000	1 to 900	1 to 800	1 to 700	1 to 600	1 to 500	1 to 400	1 to 300	1 to 200	1 to 100
2.5	+	+	+	+	+	+	+	+	—	—
5.0	+	+	+	+	+	+	+	+	—	—
7.5	+	+	+	+	+	+	+	+	—	—
10.0	+	+	+	+	+	+	+	+	—	—
12.5	+	+	+	+	+	+	+	+	—	—
15.0	+	+	+	+	+	+	+	+[a]	—	—

A commercial lysol with a phenol coefficient of 3.3, manufactured only by Lysol, Inc., 635 Greenwich Street, New York, N. Y., was used in the foregoing test. The first test, as shown above, gave negative results at a dilution of 1 to 200 at all exposures. A second test with lysol gave negative results at a dilution of 1 to 300 at exposures of 12.5 and 15 minutes. It is apparently quite safe then to use a 1 per cent or 1.5 per cent solution of lysol as a disinfectant against the red-stripe organism.

Table IV

Results of Exposure of the Red-Stripe Organism in Dilutions of Copper Sulphate, Tests Made June 23, 1924, and Observed June 28, 1924.

Length of exposure	Dilutions									
Minutes	1 to 500	1 to 400	1 to 300	1 to 200	1 to 100	1 to 50	1 to 40	1 to 30	1 to 20	1 to 10
2.5	+	+	+	+	+	+	+	+	—	—
5.0	+	+	+	+	+	+	+	+	—	—
7.5	+	+	+	+	+	+	+	+	—	—
10.0	+	+	+	+	+	+	+	+	—	—
12.5	+	+	+	+	+	+	+	+	—	—
15.0	+	+	+	+	+	+	+[b]	+	—	—

(a) Tube tested for red-stripe bacteria on nitrate agar, June 10, 1924; positive June 11, 1924.
(b) Tube tested for red-stripe bacteria on nitrate agar June 28, 1924; positive June 29, 1924.

There was considerable difficulty in the case of copper sulphate as a disinfectant in securing two or more tests to agree out of the six tests that were made. The first two tests, with dilutions varying from 1 to 50,000 to 1 to 50, gave positive results at all exposures. The third and fourth tests gave negative results at dilutions of 1 to 20 at all exposures, but differed somewhat at lower concentrations. The table above represents the fifth and sixth tests, which agreed with very little variation; here a dilution of 1 to 20 was sufficiently strong to kill the organism at all exposures, while positive results were observed in a 1 to 30 dilution, or less, at all exposures. From these results, copper sulphate apparently is a very poor disinfectant against the red-stripe organism, and such a solution, strong enough to kill the organism, would have a toxic effect on foliage. It is most interesting to note the toxic power of a commercial quicklime solution as compared to copper sulphate; this is brought out in Table VI.

TABLE V

Results of Exposures of the Red-Stripe Organism in Dilutions of Formalin. Tests Made June 17, 1924, and Observed June 22, 1924.

Length of exposure	Dilutions									
Minutes	1 to 180	1 to 160	1 to 140	1 to 120	1 to 100	1 to 80	1 to 60	1 to 40	1 to 20	1 to 10
2.5	+	+	+	+	+	+	+	+[a]	—	—
5.0	+	+	+	+	+	+	—	—	—	—
7.5	+	+	+	+	+	+	—	—	—	—
10.0	+	+	+	+	+	—	—	—	—	—
12.5	+	+	+	+	+	—	—	—	—	—
15.0	+	+	+	+	+	—	—	—	—	—

A slight variation occurred in the two tests made with formalin. The above dilutions were made from a 40 per cent commercial solution of formalin. Negative results were observed in the first test at dilutions of: 1 to 10, 1 to 20, and 1 to 40 at all exposures; 1 to 60, 1 to 80 and 1 to 100 at exposures of 5 minutes or greater, and at 1 to 120 for an exposure of 10 minutes or greater. Positive results in the second test, as recorded above, were observed at dilutions of 1 to 40 and 1 to 60 for 2.5 minutes. At 1 to 80, growth appeared at exposures of 2.5, 5 and 7.5 minutes, while a dilution of 1 to 100 gave positive results at all exposures. It would not be well to use less than a 1 to 20 solution of formalin as a disinfectant for the red-stripe bacteria. A 4 per cent formalin solution is generally used for preservation of specimens, and such a strength would easily destroy all organisms present after an exposure of 5 minutes or more. This low strength, however, could not be recommended for field use with any degree of safety.

(a) Tube tested for red-stripe bacteria on nitrate agar, June 22, 1924; positive June 23, 1924.

Table VI

Results of Exposures of the Red-Stripe Organism in Dilutions of Commercial Quicklime in Water. Tests Made June 13, 1924, and Observed June 18, 1924.

Length of exposure	Dilutions in terms of per cent									
Minutes	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
2.5	+	+	+	+	+	+	+	+[a]	—	—
5.0	+	+	+	+	+	+	—	-	-	—
7.5	+	+	+	+	+	- -	—	—	—	—
10.0	+	+	+	+	+	-	—	—	—	—
12.5	+	+	+	+	+	—	—	—	—	—
15.0	+	+	+	+	+	—	—	—	—	—

A 10 per cent stock solution was made by slaking 100 grams of commercial quicklime, which contained 71 per cent available calcium oxide, and made up to 1,000 cubic centimeters with distilled water; the above dilutions were made from this 10 per cent stock solution. The first test, as shown in Table VI, gave negative results at: 0.09 per cent at all exposures, 0.08 per cent and 0.07 per cent for 5 minutes or longer, 0.06 per cent at exposures of 7.5 minutes or longer, 0.05 per cent for 10, 12.5 and 15 minutes, and at 0.04 per cent at an exposure of 15 minutes. In a second test a slight difference was noted from the first, in that growth occurred in a dilution of 0.04 per cent at all exposures; otherwise the results of both tests were identical.

Usually in plant-disease studies copper sulphate has been considered in much the same class as mercuric bichloride as a fungicide and bactericide. The foregoing tests show the extremely low toxicity of copper sulphate against the red-stripe bacteria, and are in close agreement with the results with *Pseudomonas citri* as shown by Lee.[2] In contrast to the low toxicity of copper sulphate is this very high bactericidal action of limewater prepared from commercial quicklime. In this, also, the present results are in close agreement with the results of Lee with limewater against the citrus-canker organism.

Table VII

Results of Exposures of the Red-Stripe Organism in Dilutions of Lime Sulphur. Tests Made June 9, 1924, and Observed June 14, 1924.

Length of exposure	Dilutions									
Minutes	1 to 200,000....	1 to 100,000....	1 to 50,000.....	1 to 20,000.....	1 to 10,000.....	1 to 5,000.....	1 to 2,000.....	1 to 1,000.....	1 to 500.....	1 to 100.....
2.5	+	+	+	+	+	+	+	+	+	—
5.0	+	+	+	+	+	+	+	+	+	—
7.5	+	+	+	+	+	+	+	+	+	—
10.0	+	+	+	+	+	+	+	+	+	—
12.5	+	+	+	+	+	+	+	+	+	—
15.0	+	+	+	+	+	+	+	+	+ [b]	- -

(a) Tube tested for red-stripe bacteria on nitrate agar June 18, 1924; positive June 19, 1924.
(b) Tube tested for red-stripe bacteria on nitrate agar June 14, 1924; positive June 15, 1924.

A liquid lime sulphur was made up to a density of 32° Beaume and dilutions were made from this stock solution as indicated in Table VII. Negative results in the first test were observed in a dilution of 1 to 100 at all exposures, while positive results were noted in a 1 to 500 solution at all exposures. This test was repeated and the results were identical with those of the first test. Spraying is the general method used for applying liquid lime sulphur, and its use against red-stripe disease might be desirable in cases where a few valuable plants or even a very small area is to be protected.

TABLE VIII

Results of Exposures of the Red-Stripe Organism in Dilutions of Uspulun. Tests Made June 13, 1924, and Observed June 18, 1924.

Length of exposure	Dilutions									
Minutes	1 to 1,000	1 to 900	1 to 800	1 to 700	1 to 600	1 to 500	1 to 400	1 to 300	1 to 200	1 to 100
2.5	+	+	+	+	+[a]	—	—	—	—	—
5.0	+	+	+	+	—	—	—	—	—	—
7.5	+	+	+	+	—	—	—	—	—	—
10.0	+	+	+	—	—	—	—	—	—	—
12.5	+	+	—	—	—	—	—	—	—	—
15.0	+	+	—	—	—	—	—	—	—	—

Uspulun is a seed disinfectant manufactured by the Bayer Company, Inc., New York, N. Y. Claims are also made for this disinfectant that it stimulates the germination of seed. Two preliminary tests were made with uspulun in order to determine the range of its toxic action against the red-stripe organism. The first test with dilutions from 0.5 per cent to 5 per cent gave negative results, while the second test with dilutions from 1 to 1,000,000 to 1 to 1,000 all gave positive results; the third and fourth tests with the above dilutions agreed very closely. A 1 to 500 solution killed the organism at all exposures in both the later tests.

TABLE IX

Results of Exposures of the Red-Stripe Organism in Dilutions of Dupont Fungicide No. 1. Tests Made June 19, 1924, and Observed June 24, 1924.

Length of exposure	Dilutions in terms of per cent									
Minutes	0.01	0.05	0.1	0.5	1.0	2.0	4.0	6.0	8.0	10.0
2.5	+	+[b]	—	—	—	—	—	—	—	—
5.0	+	+	—	—	—	—	—	—	—	—
7.5	+	+	—	—	—	—	—	—	—	—
10.0	+	—	—	—	—	—	—	—	—	—
12.5	+	—	—	—	—	—	—	—	—	—
15.0	+	—	—	—	—	—	—	—	—	—

(a) Tube tested for red-stripe bacteria on nitrate agar June 18, 1924; positive June 19, 1924.
(b) Tube tested for red-stripe bacteria on nitrate agar June 24, 1924; positive June 25, 1924.

Dupont Fungicide No. 1 is manufactured in the form of a very finely divided powder by E. I. du Pont de Nemours & Company, Wilmington, Delaware. The dilutions used, as shown in Table IX, were made from a 10 per cent stock solution consisting of 10 grams of the powder in 100 cubic centimeters of sterile distilled water. Two tests were made and the results agreed very closely. A dilution as low as 0.1 per cent gave negative results at all exposures, while a 0.05 per cent solution gave negative results at exposures of 10 minutes or greater. This fungicide has a very high toxic action on the red-stripe organism. It is quite possible that this fungicide may be used in the form of a dust against many of the common plant diseases with success.

Summary

In the absence of organic matter, the red-stripe organism is killed by phenol at a dilution of 1 per cent at an exposure of 5 minutes and longer. Compared with *Bacillus typhosus*, the test organism used by Anderson and McClintic, this organism is slightly more resistant to the action of phenol. There is this difference, however, that the tests with *B. typhosus* were made with one-day-old cultures, while it has been necessary to use three-day-old cultures of the red-stripe bacteria; the slightly greater resistance to phenol of the red-stripe organism could, therefore, be attributed possibly to the greater age of the cultures used. This organism is also slightly more resistant to phenol than the organism of citrus canker, *Pseudomonas citri*, tested by identical methods.

The red-stripe organism was killed by mercuric bichloride at a dilution of 1 to 20,000 at exposures of 2.5 minutes and longer; by lysol, at a dilution of 1 to 200 for 2½ minutes exposure and longer; by copper sulphate at a dilution of 1 to 20 for exposures of 2½ minutes or longer; by formalin at a dilution of 1 to 20 for exposures of 2½ minutes, and at 1 to 40 for exposures of 5 minutes or longer; by solutions of commercial quicklime at a dilution of 0.09 per cent for exposures of 2½ minutes and 0.08 and 0.07 per cent at exposures of 5 minutes or longer; by lime sulphur at a dilution of 1 to 100 for exposures of 2½ minutes; by the Bayer preparation, called Uspulun, at a dilution of 1 to 500 for exposures of 2½ minutes, and at 1 to 600 for exposures of 5 minutes or longer. A preparation manufactured by the du Pont de Nemours Company, known as Dupont fungicide No. 1, killed the organism at a dilution of 0.1 per cent for exposures of 2½ minutes, and at 0.05 per cent for exposures of 10 minutes or more.

In the application of disinfectants, their toxicity is frequently lessened in the presence of organic material. The results which have been presented in this paper, having been in the absence of organic matter, can be considered only as showing the comparative value of the disinfectants. Experimental results in the presence of organic matter would be upon an even more arbitrary basis, since the quality and quantity of the organic matter would introduce two variables.

In the field, the results obtained from these tests should be made the criteria, and where red-stripe bacteria are known to be in the presence of organic matter, the use of discretion in the concentration of the disinfectants applied will be necessary.

Allowing for a factor of safety, from the results presented here, the following dilutions of each disinfectant tested can be recommended: phenol 2.5 per cent; mercuric bichloride, 1 to 10,000; lysol, 1.5 per cent; copper sulphate, 10 per cent; formalin, 10 per cent; 0.2 per cent solution of quicklime; lime-sulphur, 1 to 50; Uspulun, 0.5 per cent; Dupont fungicide No. 1, 0.2 per cent. For field use, should eradication ever become necessary, the use of mercuric bichloride would seem most desirable because of its high degree of toxicity to the red-stripe organism and its comparatively low cost. The very strong action of Dupont fungicide No. 1 is of possible future interest in case attempts at field methods of control of the disease should ever be desirable.

The writer wishes to express his appreciation to H. Atherton Lee, Pathologist of the Experiment Station of the Hawaiian Sugar Planters' Association, for his valuable suggestions and assistance.

Transmission of Red-Stripe Disease by Cane Cuttings

By H. Atherton Lee

An outbreak of red-stripe disease occurred on the island of Oahu in a small planting of Kohala seedling varieties, grown from cane cuttings originating in Kohala. This outbreak, together with the knowledge of other cane diseases of a similar nature, has led to the belief that red stripe is transmitted, in some cases at least, by cuttings from diseased plants.

To prove or disprove this point an experiment was undertaken in the Kohala district, in which cuttings from healthy Yellow Tip cane were planted against cuttings from Yellow Tip cane badly affected with red-stripe disease.

The First Experiment

Five hundred cuttings were selected from plants of Yellow Tip cane which were entirely free from the smallest lesion of red-stripe disease. Five hundred cuttings were then selected from cane stalks badly affected with red-stripe disease. In these cuttings an effort was made to secure top seed in which the red-stripe infection had run down through the central cylinder of leaves, into the top of the cane itself. In other words, the cane stalk was affected as well as the leaves. It may be of interest to note, in passing, that there was no difficulty in securing such diseased cuttings, but it required a considerable search to find the cuttings from stalks entirely free of the disease.

Four hundred of the healthy cuttings were planted in 2 plots of 4 rows to each plot and 50 cuttings to each row. These plots were numbered 1 and 3. Alternating with these 2 plots of healthy cuttings were placed 2 plots of cuttings from cane affected with red-stripe disease, each of these 2 plots of diseased cuttings also having 4 lines each with 50 cuttings to a row; these plots were numbered 2 and 4.

A fifth plot was made of 4 lines of 50 cuttings to each line, in which healthy cuttings were planted alternating in the row with diseased cuttings. That is, in each line of this fifth plot there were 25 healthy cuttings and 25 cuttings from red-stripe plants, the healthy cuttings alternating with the diseased cuttings.

A sixth plot also was prepared of 4 lines with 50 cuttings to the line. The cuttings in this plot were selected from stalks of cane entirely free from the disease, but the knives used in cutting this seed had been previously used in cutting diseased seed. All plots were planted February 26, 1924.

Plots 2 and 4 were to test the transmission of the disease by cuttings from affected plants, and plots 1 and 3 were check plots. Plot 5 was intended to test the extent of direct transmission from diseased plants to contiguous healthy plants. Plot 6 was to test the transmission of the disease by infected cane knives.

A chart showing the layout of the experiment follows in Fig. 7:

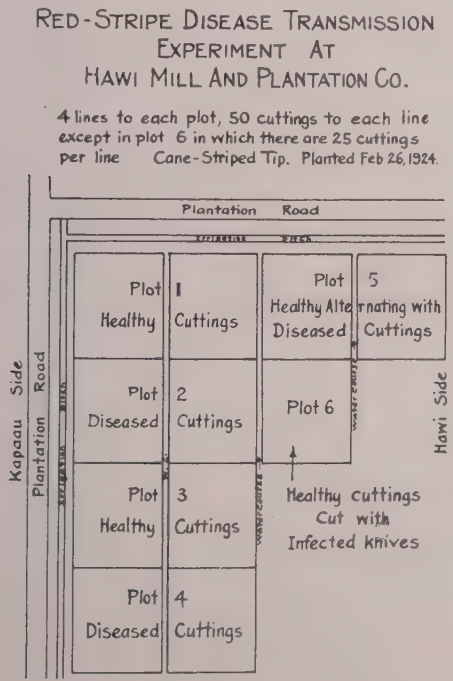

Fig. 7.

All plants germinating in all 6 plots were free of the disease on March 25, one month later. All plants in all plots remained free of the disease until an inspection was made on April 29, when 2 plants in plot 4 were found diseased. The lesions in these cases infected the leaf only and did not run down into the leaf sheath or stem. There was no visible connection between the leaf lesion and the cuttings used as seed. The conclusion was reached that these affected plants resulted from aerial infection rather than transmission by the cuttings.

The conclusions reached from this experiment were, tentatively, that the disease was not transmitted by cuttings, nor by infected knives used in cutting the seed cane. The experiment, however, was repeated.

Second Experiment

On May 22 and 23, 1924, the foregoing experiment was repeated with the omission of the plantings of cuttings made with infected knives. Plots 1 and 3 consisted of 4 lines each of 50 cuttings to a line, all cuttings from healthy plants. Plots 2 and 4 alternated with plots 1 and 3 and consisted each of 4 lines with 50 cuttings from diseased plants to each row. Plot 5 consisted of 4 lines with 50 cuttings to each line; in this plot the 50 cuttings consisted of 25 healthy and 25 diseased, the healthy cuttings alternating with the diseased cuttings.

The arrangement of the experiment is shown in Fig. 8.

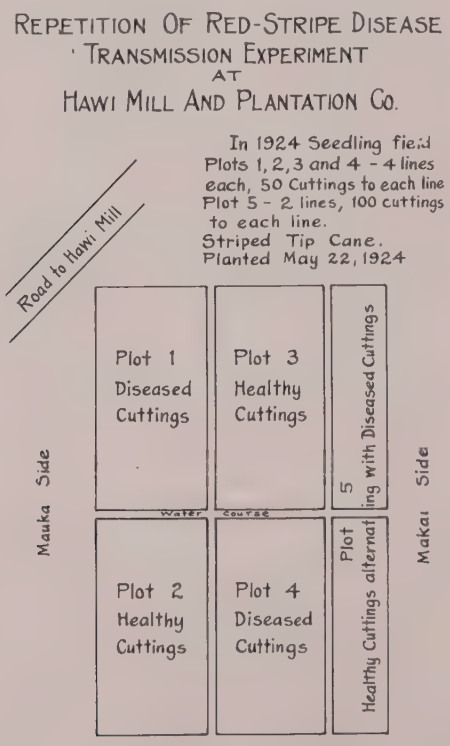

Fig. 8.

On June 27, 1924, one plant with the disease was observed by C. C. Barnum in plot 5, originating from a diseased cutting. All other plants germinating were entirely free from red-stripe disease. The affected plant showed a very clear-cut line of infection from the affected cutting up through the stem into the leaves.

Conclusions

Although a definite transmission of the infection has been shown by cuttings, such a transmission occurred in only one case from a total of 1,000 badly diseased cuttings. The apparent conclusion is, that for plantation practice in the Kohala district, the percentage of transmission by cuttings is so slight that expenditure of time in the selection of seed cuttings would not be warranted. The disease apparently is not transmitted by the use of infected knives in preparing seed cuttings, or at least such transmission occurs in only a very small percentage of cases.

The transmission of the disease in a small percentage of cases by cuttings from affected canes is clearly established, however; spread of the disease by cuttings is, therefore, possible, and adherence to the policy of no transportation of cane cuttings from Kohala into unaffected districts is still advisable.

The very small percentage of diseased plants resulting from planting infected cuttings was unexpected in the case of a bacterial disease of this nature. In the field, however, it is found that infection running down through the central cylinder into the cane very rarely passes beyond a few of the topmost joints. The red-stripe organism seems to cause a leaf disease primarily, and a top rot of the cane in a fairly large percentage of cases, but does not affect the cane stalk to any extent. Moreover, the disease is not one of the vascular system; the organism is found more in the parenchyma and sheath cells than in the phloem or xylem. These points could readily account for the very small percentage of diseased cases resulting from planting cuttings from affected cane.

The experiments presented in this paper were made possible by the management of the Hawi Mill and Plantation Company; appreciation is also expressed to W. C. Jennings, Raymond Conant and C. C. Barnum for assistance with these experiments at various times.

The Susceptibility of Roots, Stalks, Leaf Sheath and Leaf Blades to Red-Stripe Disease, and the Relationship of Maturity of Tissues to Increasing Resistance to Red Stripe

By Clyde C. Barnum and J. P. Martin

Red stripe of sugar cane is primarily a leaf disease, although occasionally infection runs down through the younger leaves into the growing tip of the cane, causing a top rot. For the purpose of determining the entire extent of injury from the disease, inoculations with the causal bacteria were made in the various parts of the cane stalk, the leaf sheaths, and leaves of various degrees of maturity. These studies have yielded interesting results which were not apparent from field

observations of the disease as it occurs naturally. A better understanding has also been obtained of the measures necessary to prevent the dissemination of the disease into unaffected districts. The results of these studies are presented briefly in this paper.

Susceptibility of the Roots of the Tip Varieties

Four tests of approximately 30 roots each were made on the roots of young cane plants to determine the effect of needle-puncture inoculations with the red-stripe organism on soft, vigorously growing young root tips. The roots were first carefully exposed by digging a trench along one side of the row of cane. Clean white roots, usually 6 to 8 inches long, were selected. All other exposed roots were trimmed away. Roots on as many individual cane stools as possible were used; often only a small number of plants afforded ideal conditions for this experiment. The selected roots were always allowed to remain attached to the parent plants and were not injured in any way. Each root was washed free of soil and made absolutely clean with several washings and rinsings with sterile condensation water. Small tufts of sterile absorbent cotton were used to wash these roots. For each test run, both control and inoculated roots were used. The controls were made first, as follows: each root was pierced at 5 points, the first point was located one-half inch from the root tip, and each successive puncture one-half inch farther back on the root. A sterile inoculating needle was used for each root so punctured. When the five punctures were completed, the root was immediately wrapped in sterile absorbent cotton, wet in sterile condensation water. The roll of wet cotton containing the root was then wrapped in paraffin paper, closed at the tip end and tied firmly with string at the plant end of the paper roll. With this moisture partially sealed around each inoculated root there was little opportunity of drying. Soil was replaced around these roots during the incubation period. The hands of the operator were frequently sterilized in a 1 to 1,000 aqueous solution of mercuric bichloride and rinsed in sterile condensation water throughout all these tests. The inoculated roots were treated in the same manner, except that the punctures were made with inoculum from pure cultures of the red-stripe organism. Completed inoculations are shown in Fig. 9, which gives an idea of the methods used.

In the first two tests made on cane roots, plants were chosen which had badly infected aerial parts, an indication that these stools were highly susceptible to red-stripe disease. Previous to and during the time of these early tests, frequent rains occurred which continued to wash bacteria into the soil from the diseased aerial parts. Subsequent work on soils has proved beyond doubt that this occurs regularly during rains. For this reason the controls in these three tests were not as perfect as in some of our later work where healthy plants were used for inoculations.

First Test: In this test, 75 punctures were inoculated on 15 roots on 5 stools of Striped Tip cane. Although 6 roots died on account of unfavorable soil conditions, 53 per cent of the punctures were positive on May 7, 1924, after 7 days' incubation. On the same stools 70 control punctures were made at the same time on 14 roots. Of these punctures, 12.8 per cent were positive. The positive

Fig. 9. Photograph of inoculated and wrapped roots of Striped Tip cane, made immediately after the inoculations were completed.

controls can presumably be attributed to the leaching, by frequent rains, of bacteria from the diseased leaves above and consequent root infection. All positive lesions were definite red areas surrounding the needle punctures on the individual roots. The negative control punctures developed no red tissues at the points of puncture.

Second Test: On May 16, 65 punctures were made on 13 healthy roots on 2 large susceptible stools of Striped Tip cane. The red-stripe organism was introduced at each puncture. Of these, 21 punctures developed red lesions, giving 32 per cent positive results. Controls were made in 70 punctures on 14 roots on 3 large stools at the same time. Of these, 21 punctures or 30 per cent developed red lesions, while 4 roots were dead as a result of unfavorable soil conditions. The inoculations were all incubated a period of five days.

Third Test: Since our field tests had established the almost universal susceptibility of all Striped Tip canes when growing vigorously, the plants used for this test were chosen from plant cane growing in an irrigated garden at Hawi. These plants were, at the time these inoculations were made, free from red-stripe infection of the aerial parts, as all visibly infected leaves were carefully cut and destroyed. Heavy irrigation had induced a strong growth of clean white roots, which were exposed in digging a deep trench along the row.

The roots were prepared as described earlier in this paper, and on 13 roots, scattered along the side of the trench, 65 inoculations with the red-stripe organism were made on July 8, 1924. After 6 days incubation, 54 punctures were found positive, or a total of 83 per cent of the inoculated punctures had developed red lesions. No further terminal growth of roots had occurred in these inoculated roots, and only a very weak lateral root growth had been made on a few roots. Many roots were killed by the infection, the entire root tissues being red. The

control roots were punctured with sterile needles and wrapped as in other tests. For controls, 65 punctures were made on 13 roots, which resulted in only 4 definitely positive lesions. Two additional lesions were considered doubtful. The controls, therefore, developed only 9.2 per cent positive lesions in 7 days' incubation. The control roots had in all cases continued to grow, had perforated the paraffin paper wrappers and extended usually several inches beyond. After this examination of these roots the soil was replaced around the roots and wet down. After the tenth day of incubation the roots were again examined. It was found that all of the inoculated roots were killed and rotted at this time and that practically all of the control roots were still growing well. Both the inoculated and control roots were photographed at this time and the reproduction is shown in Fig. 10.

Fig. 10. On the left are control shoots showing continued growth of new white tissues. On the right are inoculated roots showing absence of new growth. These roots were cut away from the plants after ten days' inoculation.

Fourth Test: Plant cane growing in the garden under irrigated conditions was used for this test. At this time there was a liberal amount of infected leaf material on all of the plants used. On October 6, two long trenches were dug along two rows of cane and a sufficient number of healthy roots were found and

treated as before. On 15 of these roots, 75 inoculated punctures were made, 5 punctures on each root, the roots wrapped and again covered with soil, as in the previous tests. After 13 days' incubation, 3 roots were found entirely rotted and red; several roots had not developed any lesions, but 20 positive inoculations were found, making the results 26.6 per cent positive. Only 8 of these roots were making active growth at the conclusion of the test. During the time of this test heavy rains prevailed and the trench was filled with water several times. This excess amount of water may have been a factor which reduced the percentage of positive results. The control punctures were made as before on 15 healthy roots. In this case, 75 punctures were made with 15 sterile needles, and wrapped as usual. Of these 75 control punctures, 14 developed positive lesions in 13 days' time, or 18 per cent, and 13 roots had made good growth, showing that the infection was not severe.

The most serious effect on these roots appears to have been the checking of terminal root growth. The inoculated roots as a whole, when compared with the controls, showed in all tests very much less growth than did the same number of control roots; this effect is shown in Fig. 10.

TABLE I

Results of Control Punctures and Inoculations With Red-Stripe Organism Made on Roots of Striped Tip Canes

	Control Roots			Inoculated Roots		
Test No.	No. punctures made	No. punctures positive	Percentage positive	No. punctures made	No. punctures positive	Percentage positive
1.....	70	9	12.8	75	40	53.3
2.....	70	21	30.0	65	21	32.3
3.....	65	6	9.2	65	54	83.0
4.....	75	14	18.0	75	20	26.6
Totals and averages ..	280	50	18	280	135	48.0

By referring to Table I the results of these four tests may be found summarized. The average percentage of positive punctures of the 280 punctures made on the control roots is seen to be 18 per cent. Of the same number of inoculated punctures 48 per cent were positive. This higher percentage in favor of the inoculated roots indicates that direct infection on the roots is dependent upon fairly heavy inoculation, the mass inoculation with the pure culture of the organism having produced a much higher percentage of positive results than the casual soil infection, due to leaching of bacteria into the soil.

In order to establish the fact that the lesions of red tissues on the roots, at the point of puncture, were caused by the same organism which produces red stripes on the leaves of the same plant, a few of the inoculated roots were brought to the laboratory at the Station in a sealed metal container for re-isolation studies on the organism in the reddened tissues. Sections of these red root lesions were plated in the usual manner, and from the dilution plates, colonies which resem-

bled those of the red-stripe organism were fished out and planted on dextrose agar. Some of these were found to be gas producers and were immediately discarded. Transplants made of three promising colonies on nitrate agar were found to reduce nitrates to nitrites, a characteristic reaction of the red-stripe organism. Healthy, young, growing shoots of Striped Tip cane were obtained and placed in beakers containing water to keep the leaves fresh. Using the three nitrate-reducing colonies just obtained, inoculations were made on three of the cut shoots of cane in the culture room at the laboratory. Ten punctures were made with a needle, with inoculum from one of the three cultures, on the two youngest leaves on one shoot. The other two cultures were inoculated in the same manner on similar stalks, tags were attached to each stalk and a control stalk was punctured in 10 places with a sterile needle. A bell jar was then inverted over the stalks, and after 7 days' incubation 28 punctures were positive on the inoculated stalks and none were positive on the control stalk. A stripe, one inch long, developed on one leaf that had been inoculated.

The re-isolation of the causal organism from the roots and the inoculation of the susceptible leaves of Tip cane, producing typical lesions of red-stripe disease on these leaves, indicate very clearly that the red lesions on the roots are caused by the red-stripe organism.

Susceptibility of Striped Tip Cane Stalks

In order to determine the susceptibility of the stalks of Tip cane to red-stripe infection, three tests were made during the summer of 1924 on field cane in the Kohala district. Pure cultures of the red-stripe organism were used as a source of inoculum. The inoculations were made directly into the stalk by means of needle punctures one-fourth of an inch deep. The stalks used for these tests were free of disease, stood about 6 feet high and were growing vigorously. The leaves were stripped from the stalks, exposing in every case one young white uncolored internode and all internodes below it. On each stalk inoculated, three internodes were punctured. Two punctures were made at opposite ends of each internode, offset sufficiently to avoid convergence of stripes in a vertical plane. The first white internode, the third internode below the white one and an old colored internode (the fifth below the first white one), were inoculated on each stalk. After the punctures were made and the inoculum placed in them, the inoculated internodes were moistened with cotton which had been wet in tap water, and the wet cotton tied to each such internode. Clean paraffin paper was then wrapped carefully and tightly around the inoculated portion of the stalk and newspaper wrapped and tied about the paraffin paper. This prevented excessive drying and excluded the sunlight.

First Test: Inoculations were made as described above on April 30, 1924, on Striped Tip canes. Ten stalks were inoculated, making 60 punctures in all. On May 10, 90 per cent of these punctures were positive, having produced long red lesions. The lesions which developed at the points of puncture were often 50 mm. in length and 5 to 10 mm. wide. The longest and most pronounced lesions were found in the youngest or white internodes. The lesions usually extended as much as 12 mm. deep into the inner tissues.

Second Test: Ten stalks each of controls and inoculated stalks were put in June 26, 1924. Of the 60 punctures made with the red-stripe organism, 50 were found positive on July 8, making a total of 83 per cent positive results. Controls were made with a sterile needle and put in at the same time. Of these 60 control punctures, 20 became active lesions after 12 days' incubation, or 33.3 per cent positive. During the period of incubation the stalks were repeatedly moistened and frequent showers occurred, all of which may have been instrumental in washing air-borne bacteria of red-stripe disease into the punctures. This would doubtless account for the high percentage of positive results on the controls.

Third Test: Late in July, 1924, controls and inoculations were again put in on cane in the same field. Ten stalks with six punctures each were punctured in each series. After 16 days' incubation the wrappers were removed and the stalks examined. From 60 inoculated punctures 48 positive lesions developed, making a total of 80 per cent positive results. The 60 controls yielded only 8 positive lesions, or a total of 13 per cent positive results. In making these punctures great care was exercised to prevent contamination. Sterile condensation water was used to moisten the cotton used to keep the punctures moist, and the hands were carefully disinfected each time a stalk was inoculated and wrapped. Stalks were used which had no sign of leaf infection in order to minimize the contamination or infection of the control punctures. None of the lesions which developed on the controls were as pronounced as those on the inoculated stalks. In Table II is found a summary of the results obtained.

TABLE II

Results of Three Series of Controls and Inoculations, Each Series Made on Ten Stalks of Striped Tip Cane

	Control Stalks			Inoculated Stalks		
Test No.	No. punctures made	No. punctures positive	Percentage positive results	No. punctures made	No. punctures positive	Percentage positive results
1.....	..	..	....	60	54	90
2.....	60	20	33.3	60	50	83
3.....	60	8	13	60	48	80
Totals and averages ..	120	28	23.3	180	152	84.5

Although there can be no doubt that the red-stripe organism can produce rotting of the stalks, it is only in rare cases, such as top rotted stalks and heavily inoculated canes, that a general decay of the stalk takes place. In this experimental work a few cases of badly rotted stalks were found, but the usual lesion was more or less confined to the rind of the cane, seldom extending far into the adjoining internode. Throughout the tests the white internode at the top was the most susceptible and produced the largest and longest lesions. In cases of top rot the rind is frequently the least involved, while the ground tissue may be bright red with the disease. The character of the rot of the upper internode is shown in Fig. 11. This experimental work, therefore, proves that such diseased tissues are due to invasion by the red-stripe organism.

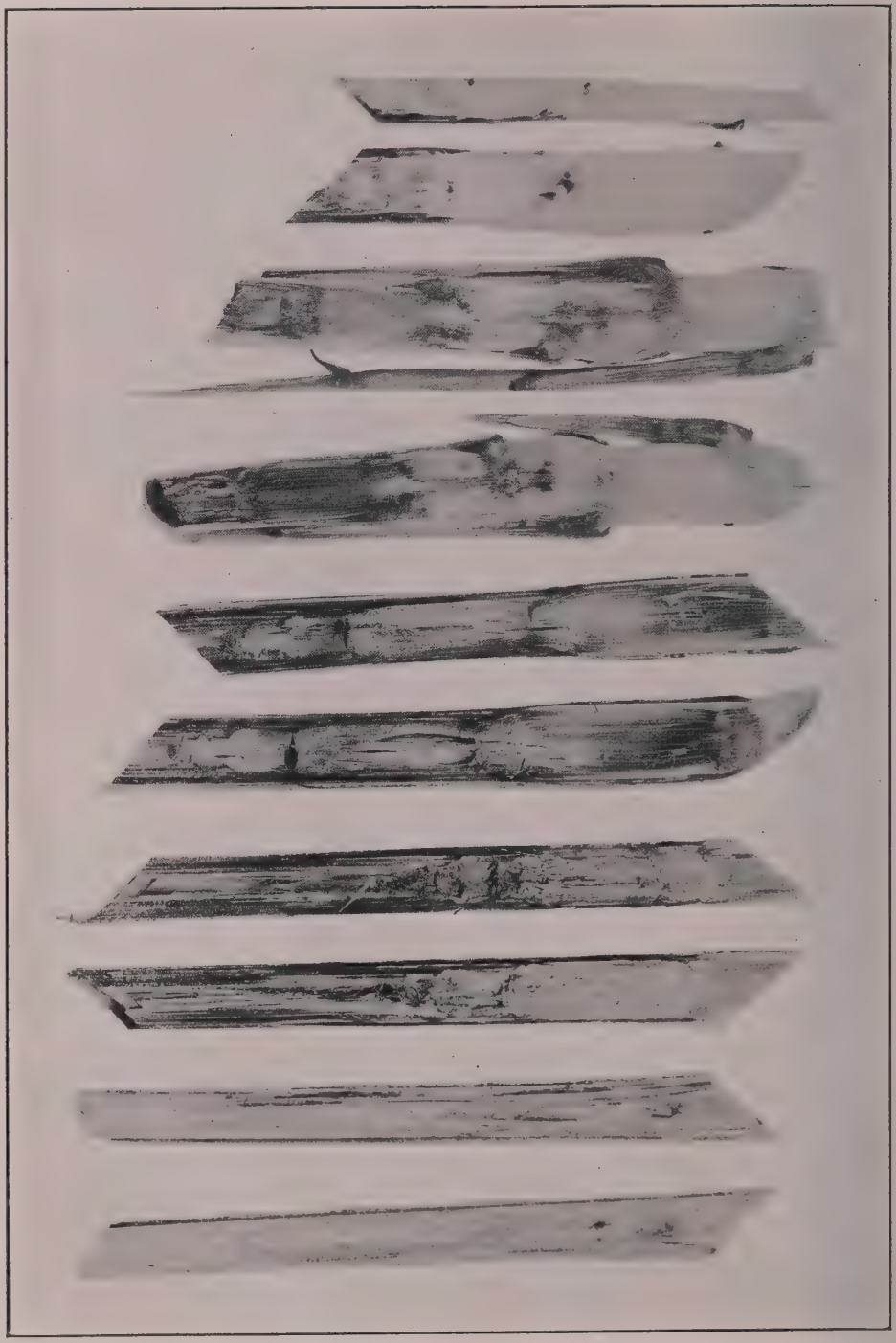

Fig. 11. Various stages of top rot of Tip canes in longitudinal sections. The dark regions were dark red at the time the material was photographed.

Susceptibility of Leaf Sheaths

Inoculations were made early in the summer of 1924 on the healthy young leaf sheaths attached to the upper node of the youngest white internodes used in the stalk inoculations. Only one leaf sheath on each stalk was inoculated. A diagonal row of five punctured inoculations was made in each sheath so inoculated, using pure cultures of the red-stripe organism. This work was done at the same time as the stalk inoculations; the leaf sheath was moistened, wet cotton tied in place on the sheath and it was wrapped in paraffin paper and newspaper in the same operation as the stalks so wrapped. None of the punctures made in the sheath lay in the same vertical plane, thus avoiding coincidence of stripes. Ten sheaths were inoculated on April 30, 1924, and ten days later 73 per cent of the inoculations were positive. Those inoculations made near the edges of the sheaths, in very thin tissues were usually negative, while those in the fleshy portion near the middle region of the sheath became very definite red lesions, one-fourth inch wide and extending nearly one inch, both above and below the point of inoculation. This experiment showed that definite red lesions could be produced in the fleshy parts of the sheath with the organism of red stripe, proving the susceptibility of these parts of the cane plant to the disease.

Susceptibility of the Leaf Blades

The leaves are the most commonly diseased parts of the canes in the field under natural conditions. A more complete study of susceptibility of cane leaves was carried out, therefore, than was made of the other parts of the plant.

Test of Paper-wrapped as Compared to Unwrapped Inoculated Leaves

It was indicated early in the season that the best results in inoculation work on the leaves were obtained by the use of wrappers to exclude the sunlight and maintain optimum moisture conditions on the inoculated parts. Using pure cultures of the red-stripe organism, inoculations by needle puncture were made on the 2 youngest leaves on each of 20 thrifty young cane stalks. Five punctures were made in a diagonal line, on each inoculated leaf. All the leaves were then moistened with tap water. Ten stalks were not wrapped, while ten stalks and the inoculated leaves thereon were carefully wrapped in thick paraffin paper with wet cotton inserted between the leaves. The wrapper usually enclosed 4 or 5 leaves at the top of each stalk. A single sheet of newspaper was then wrapped around the paraffin paper to exclude light, and the wrapping was then tied firmly to the stalk below the inoculated leaves and loosely at the top so that some light, air and occasional rain would be admitted. After thirteen days' incubation the results obtained from the exposed inoculated leaves showed an average of 72 per cent positive results, while the wrapped inoculations yielded 90 per cent positive results. During the time this test was in progress, a repetition of the experiment was made on similar plants in the same field. The results of the second test after ten days' incubation yielded 55 per cent positive results for the exposed inoculations, and 91 per cent for those which were wrapped in paper. Since

laboratory tests have shown that the red-stripe organism is very easily killed by drying and exposure to sunlight, these results obtained in the field substantiate the laboratory findings very closely. The higher percentage of positive inoculations obtained under the wrappings indicated that natural infection of cane leaves was favored by damp, cloudy weather extending over periods of several days. The lower results obtained on the exposed, not wrapped leaves, indicated that infection took place in direct sunlight and in punctured inoculations of the leaves, but much less than where protection was given the leaves.

As a result of the findings of this experiment all subsequent inoculations of cane leaves with the red-stripe organism were protected with both paraffin and newspaper wrappers, with moist cotton inserted between the leaves.

Relative Susceptibility of Leaves of Various Ages on the Same Stalks

Having determined the optimum conditions favoring successful inoculations of the leaves, an experiment was made to determine the relative susceptibility of leaves of various ages on the same stalks. For this work three leaves on each stalk were inoculated, making five punctures in each leaf, with an inoculating needle bearing inoculum from pure cultures of the red-stripe organism directly into the upper leaf surface. The five punctures were made in a diagonal row across the leaf from side to side with the middle puncture directly in the midrib of each leaf. Moistened cotton was inserted and the leaves wrapped as previously described in this paper. Tags were attached to each inoculated stalk throughout all the tests. The various aged leaves tested were as follows: the youngest unrolled leaf of the stalk, the next oldest leaf, and the fourth leaf from the youngest. Ten stalks were inoculated, three leaves on each stalk, making a total of 150 punctures. After 8 days' incubation the results were noted as follows: the youngest leaves yielded 100 per cent positive results, the second leaves or next oldest yielded 92 per cent positive results, while the fourth leaf on each stalk yielded only 52 per cent positive results. After 17 days' incubation, during which time the wrappers had been maintained in place, but no further moisture had been added, the results were proportionately lower as a result of the drying of the tissues at the points of puncture and the leaf injury due to the exclusion of light. The later results, however, indicated most conclusively that the younger leaves were the most susceptible. This test was repeated two months later on similar cane, in the same field, which was not irrigated and was not making a vigorous growth. The results obtained were absolutely negative on account of the poor growing condition of the cane. At this time the soil was too dry to favor growth and the leaf tissues were not susceptible to the bacteria.

A third test was, therefore, made on young plant cane which was frequently irrigated and was, as a result, in a growing condition. In this test only two leaves were inoculated on each of the ten stalks tested. The youngest leaf and the sixth leaf from the youngest were inoculated on each stalk. The older leaves did not stand the wrapping and exclusion of light so well as the younger leaves. Four of the older leaves were dead at the close of the test. After 10 days' incubation the results were as follows: the younger leaves yielded 82 per cent positive results, while the older leaves gave only 30 per cent positive results. The lesions result-

ing on the younger leaves were always much more pronounced than those developing on the older leaves. This condition was noted throughout all the inoculations made during the season.

A fourth test was made while the third test was in progress. In this test three leaves were inoculated on each of ten stalks of vigorously growing Tip canes. The youngest leaf, the next oldest leaf, and the sixth leaf from the youngest were inoculated, each with 5 punctures on each leaf. After 9 days' incubation in wrappers the results were as follows: the youngest leaves yielded 82 per cent positive results; the second leaves, or next oldest, yielded 82 per cent, while the sixth oldest leaves yielded only 52 per cent positive results.

A summarized table of results of the four tests appears as Table III. The results, as shown in the table, indicate very conclusively that the younger leaves are by far the most susceptible on the cane plant under the most favorable conditions for infection.

TABLE III

Results Obtained in Four Tests of Relative Susceptibility of Cane Leaves of Various Ages, Expressed in Percentages

No. of test	Youngest leaves	Second leaves	Oldest leaves	Age of oldest leaves; position on stalk
1.....	100	92	52	4th
2.....	0	0	0	4th
3.....	82	0	30	6th
4.....	82	82	52	6th
Averages.....	88	87	44	

These results are derived from tests in which a total of 500 punctures were made. The tests cover a period of nearly three months during the summer season of 1924.

The Relationship of Maturity of Cane Plants to Increasing Resistance to Red-Stripe Disease

Having shown that the relative age of leaves was a factor in the susceptibility of cane parts, a test was made to determine the susceptibility of the two youngest leaves on stalks of different ages growing under, as nearly as possible, identical soil conditions; or, in other words, tall stalks as compared to young short stalks.

In the sprinkler field at Hawi Mill & Plantation Company, where ideal moisture conditions prevailed, a few rows of Tip cane had been cut for body seed during the summer of 1924. These rows soon produced a vigorous ratoon growth, while the adjacent rows of Tip cane continued to grow, being then about 6 to 8 feet tall. Ten young shoots were selected on the row of ratoons, and the two youngest leaves on each stalk were inoculated and wrapped. In the adjoining row of tall

cane ten stalks were inoculated, the two youngest leaves on each stalk being inoculated and wrapped. In both cases after ten days' incubation 97 per cent positive results were obtained. The total length of all stripes produced on the leaves of the tall stalks was 55.5 inches. On the short stalks 63.5 inches of characteristic red-stripe were produced on the inoculated leaves. This increased length of stripes is slight and does not indicate a great difference in susceptibility. The results indicate that rapidly growing leaves on old and tall stalks of cane are as susceptible to punctured inoculations as are leaves of the same age on young shoots of the same cane variety growing under similar soil conditions. This does not necessarily indicate, however, that under natural conditions of probable infection without punctures, presumambly through the stomata, the susceptibility of such differently aged stalks would be identical.

Infection Through Stomata Compared With Infection at Punctures

An investigation was undertaken to determine whether or not punctures were necessary in order to obtain leaf infection with the red-stripe organism. The first test, in April, 1924, was made on vigorously growing Tip cane. An infusion of the red-stripe organism was prepared, milky white in distilled water. This infusion was painted on the upper surfaces of the two youngest leaves on each of 20 stalks of cane. Ten of these stalks were immediately wrapped in paraffin and newspaper as before described. No punctures were intentionally made in any of these leaves. These leaves constituted the "not punctured" series of the test. The two youngest leaves on the other 10 stalks were each punctured in 5 places with a sterilized needle and wrapped as usual.

After 10 days incubation the wrappers were removed and counts made. On the 20 leaves not punctured, only 29 definite lesions had developed. On the punctured leaves 69 stripes had developed at the points of puncture, this being 69 per cent positive results from punctured inoculations. The 29 lesions on the "not punctured" leaves developed into definite red stripes. The test was repeated two months later in the same manner on young cane in the same field. On the "not punctured" leaves only 28 lesions were produced. The punctured inoculations yielded 85 per cent positive results, with 85 definite red stripes resulting. The stripes produced, following the puncturing of the leaf surface, were much more pronounced than those induced by stomatal infection. Since natural infection in the field occurs to a much higher degree of intensity than was obtained in the foregoing tests, it was assumed that the failure to produce a very large number of infections was probably due to the closing of the stomata on the leaf surfaces immediately after the leaves were wrapped in the opaque wrappers. An experiment was planned to determine, if possible, the relation of light and darkness under optimum maintained moisture conditions in tests of leaf susceptibility to red-stripe disease. For this test of leaf susceptibility, plant cane growing in the garden at Hawi, where fertilization and irrigation could be controlled, was used. Optimum soil conditions were maintained throughout the test and the

results obtained from this test are very conclusive. For this test 120 stalks of Striped Tip cane were used. An infusion, milky white in distilled water, was prepared and applied where necessary with a cotton swab to the leaf surface as designated. Throughout the test the question of light and darkness was considered at all times. Where the plants were wrapped to admit light, only one wrapping with thin paraffin paper was used. This paper was too thin to retain the growing leaves through the period of incubation and was frequently ruptured by the growing leaves. This condition permitted great aeration and consequent drying of the inoculated leaves, and must have reduced the sum total of positive results considerably. Those plants wrapped to prevent the admission of light as much as possible were not only wrapped in paraffin paper, but also in newspaper, which excluded all light except that which came down through the upper, partially open end of the cylindrical roll of paper. As may be noted in referring to the summarized table of results (Table IV), tests were made of the susceptibility of both the upper and lower leaf surfaces, separately and collectively.

TABLE IV

Showing Results Obtained From Inoculating Leaves of Striped Tip Cane With an Infusion of the Red-Stripe Organism, Incubated 10 Days Only.

Test No.	Surface of leaves inoculated	Number punctures made	Number stripes produced	Character of wrappers
S-1	None	None	1	Opaque
S-2	None	None	1	Transparent
S-3	None	100 without inoculum	2	Opaque
S-4	None	100 without inoculum	0	Transparent
S-5	Upper	None	95	Opaque
S-6	Upper	None	47	Transparent
S-7	Lower	None	85	Opaque
S-8	Lower	None	55	Transparent
S-9	Upper	100	88	Opaque
S-10	Upper	100	84	Transparent
S-11	Lower	100	75	Opaque
S-12	Lower	100	62	Transparent

Controls were run both with and without sterile needle punctures to determine the relative number of natural infections found on each plant. The results show that natural infection in this locality was quite negligible. It is quite evident that the upper and lower surfaces of the cane leaves are nearly equal in susceptibility, with a slight tendency in favor of the greater susceptibility of the upper surfaces. It is very forcibly shown that the use of opaque wrappers, which not only excluded light somewhat but did retain the moisture better than the thin wrappers of paraffin paper, favored infection of both leaf surfaces. The totals of all the stripes produced on both the upper and lower surfaces of the leaves separately are found to be approximately equal, with the one exception of the tests with punc-

tures made on the upper surfaces only. In this test the total number of stripes resulting was much higher than with the other punctured inoculations. The resulting stripes which developed on the inoculated plants were notably redder and more extensive where punctures were made than where no punctures were made.

It is very definitely shown, however, that light is not necessary in great intensity in order to develop stomatal infection on cane leaves, in so far as the wrappers used in this work are concerned. It is clearly evident that the factors which are most essential to rapid infection are those of extremely vigorous growth, the presence of soft, succulent tissues of newly formed leaves, and an optimum environment of moisture. It is evident that natural infection in the field may take place without the agency of any insects, although insect punctures are, no doubt, factors which induce a certain amount of natural infections in the field.

Summary

It is clearly shown that the roots of Tip canes are easily inoculated with pure cultures of the red-stripe organism producing red lesions; from such affected tissues the causal organism has been re-isolated and proved by etiological and pathological tests on cane leaves to be the identical organism introduced into the roots. Koch's rules of proof have been carried out completely.

It has been shown that the stalks or cane internodes are susceptible to inoculations with the red-stripe organism. The youngest internodes are the most susceptible. The ground tissue becomes a deep red, the necrotic condition finally resulting in a complete breaking down of the softer interior parts of the cane, with a decidedly putrid odor apparent when the stalk is split open. This same condition is noted in naturally occurring cases of top rot in the field. The greater resistance of the older canes and older internodes is very pronounced, resulting only in definite but restricted red streaks in most cases.

The leaf sheath is susceptible to the red-stripe organism when inoculations are made directly into the tissues of the sheath. Definite red lesions are developed under these controlled conditions. The slightest injury to the leaf sheath of Tip cane may often produce a red area that might easily be mistaken for a red-stripe lesion. The natural infection of leaf sheaths is not of great economic importance.

The young leaves of Tip cane are the most susceptible parts of the cane plant. Inoculation and infection of young leaves are favored by the use of opaque-paper wrappers which exclude light and retain applied moisture. A series of tests indicated clearly that the youngest leaves on thrifty plants were the most susceptible. Plants not growing vigorously were very resistant to the disease.

Leaf tissues of vigorously growing plants, either tall canes or short shoots, are equally susceptible to infection when inoculated with the red-stripe organism under similar environmental conditions, although field observations show that older cane is much less commonly affected than young cane.

Natural infection of the leaves occurs in the field largely through stomatal entrance of the causal organism. The upper and lower surfaces of the leaves are closely comparable in susceptibility.

The Activities of the Red-Stripe Organism in the Soil

By Clyde C. Barnum

The bacterial red-stripe disease of sugar cane has been shown to be a disease almost exclusively of Tip canes. Very few other varieties of cane are even slightly susceptible. Since the disease was found to be confined to Kohala district, a strict quarantine was placed on the movement of all sugar cane and grasses grown therein, by the Territorial Board of Agriculture and Forestry immediately after the bacterial nature of the disease was determined.

In order to more fully prevent the introduction of this disease into other districts in which Tip canes are grown, it became necessary to study all possible methods of distribution. The possibility of the natural occurrence of the causal organism in the soils of Kohala was, therefore, of considerable importance. Should the organism be found to occur in these soils and remain active over relatively long periods of time, the possibility of the distribution of the disease to other districts in Hawaii growing Tip canes, on shoes, hoofs of animals, on vehicles and agricultural implements could be ascertained, and measures taken to prevent further distribution. It was observed that on closely burned-over infected fields of Tip cane at cutting time, when all the foliage had been burned, and the entire surface of the field was black, there usually appeared, later, on the new growth, innumerable infections as soon as the young shoots had reached a height of ten or twelve inches. Whether or not this early infection, widespread in its nature, was entirely due to wind-borne bacteria from infected fields to windward, or due to local infection from the soil supporting the growing plants, was a point to be determined. These investigations were planned in order to determine the activities of the red-stripe organism in Hawaiian soils; that is, do the red-stripe bacteria live and multiply in the soil? Do they exist passively without reproduction or do they disappear in the soil?

The work was divided into three projects as follows:

1. The activity of the organism in unsterilized soil in culture tubes, as compared with its activity in sterilized soil in culture tubes.

2. The persistence of the organism in the field soils in Kohala.

3. The occurrence or absence of red-stripe infections on young plants of the Tip variety grown from healthy seed pieces planted in sterilized and unsterilized Kohala soil, inoculated with the red-stripe bacteria. All of the field work on these problems was carried out in Kohala, Hawaii.

Part I

Activity of the Organism of Red-Stripe Disease in Sterilized and Unsterilized Soil in Test Tubes

These tests were made to determine, if possible, the maximum length of time this organism could live in tubed, previously sterilized and unsterilized soil and still be capable of causing typical infection on susceptible plants. The soil used for these tests was obtained from the Experiment Station grounds in Honolulu. In all, four complete tests were made, extending over a period of approximately six months' time.

Preparation of Soil and Materials:

For each of the four tests made, the following list of materials was prepared and treated in the same manner:

One hundred and twenty tubes, each containing approximately 10 grams of soil were prepared and stoppered with cotton. Sixty of these tubes were set aside unsterilized, and sixty were autoclaved one hour each day for three consecutive days at 15 pounds pressure. One hundred and twenty wooden-handled cotton swabs were prepared, wrapped in bunches of five each. An equal number of inoculating needles were each tipped with cork and wrapped and sealed in paper. These were all autoclaved at 15 pounds pressure for one hour. Sterile cotton, sheets of paraffin paper 18 by 24 inches, and sheets of newspaper of the same size, string and labels were included in the list of materials used.

For the inoculation of the soil tubes, pure cultures of the causal organism of red-stripe disease were used. A sufficient volume of a milky infusion was made up and 4 cc. of this infusion was added to the soil in each tube of both the sterilized and unsterilized series. In inoculating the sterilized-soil tubes, care was exercised to prevent contamination. In all the tests made, the length of time after this inoculation of the tubes until the host plants had been inoculated is the period of time under consideration, that is, the length of time the organisms lived in the soil tubes.

Methods of Procedure in the Field:

To determine the presence of the organism in the tubes following the inoculation of the contained soil with infusions of the organism, vigorously growing leaves of Tip cane were inoculated from infusions made from the tubed soil, both sterilized and unsterilized soil being used each time inoculations were made. Infusions were made directly in the tubes by the addition of 10 to 15 cc. of cold condensation water from the Hawi Mill, obtained hot in sterile Erlenmeyer flasks at frequent intervals. Condensation water was the best substitute for sterile distilled water; all tap water and ditch water presumably carried the red-stripe organism and could not be used for this reason. The water was added to the

soil in the field laboratory and the tube shaken until the soil was in a completely suspended condition. Five tubes of each kind of soil were used in each instance when cane was inoculated; one cane plant was inoculated from each tube of soil. The infusion was applied to the upper surfaces of the two youngest unrolling leaves with the sterile cotton swab supplied for each tube. The punctures were made directly through this applied infusion; several short cross-scratches into the epidermis were made at each of the chosen points, followed by 3 to 5 shallow needle punctures. In the first test only 5 punctured areas or "punctures" were made on each inoculated leaf, in a diagonal row across the leaf. In the other tests, 10 "punctures" were made on each inoculated leaf, in 2 diagonal rows. Wet cotton, saturated in sterile condensation water was applied between the oldest inoculated leaf and the next oldest leaf on the plant in order to avoid restraint of new growth, and the stalk and leaves were then wrapped in clean paraffin paper so that the inoculated leaves were well protected from sunlight and drying, and newspaper was wrapped snugly around the paraffin paper. The wrappers were tied tightly with string at the lower end and loosely at the top to admit light and air. Tags were attached to each stalk, bearing the serial number of the inoculation, the date and other essential points. After ten days' incubation the wrappers were removed and examination of the inoculations made. In all cases where results were noted as positive at the "puncture," a definite red lesion had developed around the point of infection. Often the characteristic red stripe of 10 to 20 inches in length had resulted. All negative "punctures" lacked the red tissues forming the lesion and were usually of a light straw color, the result of the mechanical injury solely.

Precautions and Antiseptics Used:

In the first test of this work 2 per cent lysol followed by wood alcohol full strength were used to wash the hands and shoes of the operators. Following the results of the investigations carried on by J. P. Martin, of this laboratory, on antiseptics, in the last three tests a 1 to 1,000 aqueous solution of mercuric bichloride was used for the hands and shoes, and the hands dried on paper towelling immediately afterward. Results obtained in the early part of Test III indicated that the small amount of the mercuric salt remaining in the pores of the hands produced a toxic effect on the bacteria in the applied infusion on the leaf surface. Although each infusion was applied with a clean swab, the fingers of the left hand were frequently in contact with the infusion in the work of inoculation. Accordingly, in subsequent experiments the hands were rinsed each time in sterile condensation water and dried on sterile paper towels after using the disinfectant. This change was beneficial and seemed to indicate that the organism is extremely sensitive to mercury salts. In the inoculation work in the field, the hands of both operators were washed in this antiseptic and rinsed in distilled water before the soil tubes were opened for making the infusion, and after each stalk was inoculated, throughout each test made.

Results of Tests:

In the first test, 11 series of leaf inoculations were made, at different intervals of time, up to those on the 15th day following inoculation of the tubes with the bacterial infusion. On all inoculations made from sterilized soil, positive results were obtained. This indicated that the organism lived for 15 days in sterilized soil, producing 92 per cent positive results from inoculations made on the 12th day. Inoculations made from unsterilized soil tubes on the 12th day indicated the presence of the organism. These inoculations on the 12th day produced 76 per cent positive results. On the 15th day, from unsterilized tubes there were no positive results, indicating the reduction of the red-stripe bacteria in the unsterilized soil. Positive results from the sterilized tubes indicated the presence of the organism in the sterilized soil.

In the second test, made in June, 1924, infections were obtained from the sterilized soil tubes, yielding 7 per cent positive results as late as the 27th day, 5 per cent on the 31st day, and 1 per cent on the 34th day, indicating the gradual diminution of numbers of bacteria present in this sterilized soil. Unsterilized soil tubes yielded positive results as late as the 27th day. On the 15th day 13 per cent positive results were obtained from unsterilized soil. Results gradually dropped, becoming entirely negative on the 31st day.

In the third test, made in July and August, 1924, positive results were obtained from inoculations made on cane plants from infusions of sterilized soil inoculated 37 days previously. Even as late as this, 26 per cent positive results were obtained. Unsterilized soil infusions inoculated 37 days previously yielded 8 per cent positive results at the same time. These tests were continued even later than this, but the cane plants were not in a susceptible condition, due to lack of soil moisture, and presumably the later inoculations were totally negative for this reason. It would be very doubtful that inoculations made on the 41st day would drop off to totally negative results from both types of soil 4 days later, if it were not due to the unfavorable condition of the host plants, known to exist at that period.

In the fourth test, made in August and September, 45 per cent positive results were obtained from sterilized soil tubes inoculated 41 days previously. Unsterilized soil tubes inoculated 31 days previously yielded only 2 per cent positive results. Entirely negative results were obtained from unsterilized soil tubes 41 days old. At different periods throughout this test the cane plants suffered for want of irrigation, and the results were consequently negative until the stimulating effect of the delayed irrigation was noted on the cane in this field. It was demonstrated early in the study of this disease that susceptibility of Tip cane leaves was in proportion to the rate of growth. Slow-growing plants were found to be notably non-susceptible.

The accompanying summarized table of results for the four tests, Table I, indicates the relative longevity of the organism in the two preparations of soil. The fact is evident that this organism may live in soil, even in competition with the native soil flora, for a period of 27 days and be active enough to induce in-

TABLE I

Summarized Table of Results of Four Tests Showing the Results from Inoculations on Cane Leaves, Made from Soil Infusions of Both Sterilized and Unsterilized Soils Previously Inoculated With the Organism of Red-Stripe Disease

No. of days since tubes were inoculated	TEST NO. I Percentage positive		TEST NO. II Percentage positive		TEST NO. III Percentage positive		TEST NO. IV Percentage positive	
	Sterilized soil	Unsterilized soil	Sterilized soil	Unsterilized soil	Sterilized soil	Unsterilized soil	Sterilized soil	Unsterilized soil
1	74	76	..	..	..	..	..	..
2	96	88	..	..	..	..	38	76
3	92	88	0	0	..	..	..	..
4	64	50	..	..	..	..	..	..
5	66	28	..	..	..	..	..	..
6	56	28	..	..	3	1	..	..
7	78	38	..	..	..	..	22	26
8	96	50	0	0	..	..	..	..
10	98	54	..	..	10	4	..	..
12	92	76	..	..	..	..	24	3
13	..	..	0	0	..	..	..	..
15	26	0	10	13	..	..	7	2
16	..	..	..	..	21	1	..	..
17	..	..	..	..	..	..	0	0
20	..	..	12	3	52	0	..	..
21	..	..	..	..	..	..	0	0
22	..	..	23	0	..	..	..	..
23	..	..	..	..	..	..	0	0
24	..	..	11	1	23	0	..	..
25	..	..	..	..	..	..	4	0
27	..	..	7	1	..	..	..	..
28	..	..	..	..	..	..	0	0
29	..	..	..	..	19	1	..	..
31	..	..	5	0	49	1	8	2
32	..	..	..	..	..	..	44	0
34	..	..	1	0	48	4	..	..
35	..	..	..	..	..	..	15	0
37	..	..	..	..	26	8	..	..
41	..	..	..	..	0	0	..	..
42	..	..	..	..	..	..	45	0
45	..	..	..	..	0	0	..	..

fection of the host plants. There is evidence to show that the organism may live even longer and be active, but there are so many negative results due to dry summer weather and lack of soil moisture at these later periods in some of the last tests, that this point could not be verified by repetition in all tests. It seems evident that in sterilized soil the organism can maintain its virulence and possibly multiply to some extent. In unsterilized soil a decided decrease of numbers and virulence of the organism was noted. This decrease can, no doubt, be attributed to the competition of soil organisms present in the unsterilized soil.

Part II

Persistence of the Organism of Red-Stripe Disease in the Field Soils of Kohala, Hawaii

In making the persistence tests on the Kohala soils, simultaneously with the tubed soil experiments, it was hoped that the results obtained from one series might be corroborated by the results from the other. In some cases this result was obtained, in part at least. In other cases positive results were not obtained over as long a period of time as was indicated in the previous series for this organism, but these negative results were often due entirely to the unfavorable soil conditions of the cane fields during the tests. Shortage of irrigation water and of labor to apply the water caused this condition frequently for short intervals during the summer. This series of experiments was attempted to determine the effect of environmental conditions in the field on the organism of red-stripe disease as it naturally occurred in the soil. The only equipment necessary for this series of tests was, for each test made, 60 water blanks, each test tube containing 10 to 15 cc. of sterile distilled water, well stoppered; 60 each of cotton swabs and inoculating needles, and 15 wooden spatulas, each wrapped in paper and all autoclaved at the same time.

Method of Procedure:

In every test made and here reported, a heavily infected group of diseased Tip cane plants was first located. Following a heavy rain, or in the absence of rain a heavy sprinkling from irrigation hose during the night, the leaves and stalks from 3 or 4 heavily infected stools were cut away and the crown of the plants pulled out with as many roots as possible early the next morning. This prevented new shoots from coming again. All surrounding cane stalks nearby were cut to the ground so local re-infection of the soil would not be possible. The soil immediately under and around the infected plants which were pulled, root and all, presumably had large numbers of bacteria washed into it during the previous night. On this assumption the tests were carried out and the results clearly established this point. The infected soil was staked and well mixed as soon as the plant material was removed. No protection was given this soil at any time.

With a sterile spatula, five small samples of this infected field soil were taken at regular time intervals and placed in water blanks. Infusions were made in these five tubes by shaking, and from such soil infusions inoculations were made immediately on the most susceptible non-infected Striped or Yellow Tip cane available. Only one stalk was inoculated from each tube of infusion; the infusion was applied with a sterile cotton swab and punctured into the upper surfaces of the three youngest unrolled leaves on the stalk with the sterile inoculating needle provided for this tube. Twenty punctures were made on each leaf in 4 diagonal rows of 5 punctures in each row, from side to side of the leaf. The rows were made as far apart as possible on the leaf, depending upon the size and age of the leaves. A rather large piece of sterile absorbent cotton, thoroughly wet in sterile condensation water, was placed between the older leaves of the stalk to insure adequate humidity in the wrappers, and all the leaves were wrapped in paraffin paper and newspaper, tied tightly at the bottom and loosely at the top, in order to admit some light and air. Tags were attached to each inoculated stalk. When very young plants were used it became necessary to tie the wrapped, inoculated stalks to stakes, to prevent injury in the wind and rain. For each time interval five tubes of infusion were used, and with these five infusions five stalks were inoculated.

Host Plants Used For Inoculation:

In Test I, two-months-old Striped Tip plant cane was used. In Test II, the same planting of canes was inoculated, although different plants were used. In Test III, Yellow Tip cane planted 3 months previously was used. Plants in the same field still farther to windward were used for Tests IV and V. There was no local natural infection on the host plants used for Tests I, III, IV or V. The cane used for Test II was subject to possible infection, but there was very little present during the test period. The marked absence of natural infection on the host plants throughout the tests indicates that the stripes which developed on the inoculated stalks were caused by the bacteria introduced from the soil infusions.

Precautions and Sanitary Methods:

Throughout the tests, the hands, shoes and leggings of both operators were washed in some disinfectant before inoculations were made, and the hands again after each tube of infusion was made or used.

In Test I, the hands were washed in both 2 per cent lysol and full strength wood alcohol, and dried on paper towels. This treatment was found to be rather severe on the hands. Following the work of Mr. Martin on disinfectants a change was made to 1 to 1,000 mercuric bichloride solution. This disinfectant proved to be very efficient. Some of the later results indicated that the positive results were reduced by the small amount of this toxic salt remaining on the hands after drying. Following this determination, the hands were washed in sterile

condensation water after each washing in the bichloride solution. This was found to check the toxic effect on the infusion during the process of inoculation. Throughout the series of tests each operator wore a pair of coveralls, washed frequently and daily exposed to direct sunshine for a period of 2 to 5 hours. The wearing of these suits prevented possible infection from the usual field clothing. These suits were worn only during the inoculation of the host plants.

Results of Inoculations Made in Five Tests:

In Test I, the 125 punctures made on the last day of the test, 17 days after natural infection of the field soil, yielded 21 positive results after the organisms had remained in the soil for this period of time. In Test II, 15 positive results were obtained from 300 punctures made through soil infusions prepared 15 days after the source of infection had been removed from the soil used for infusion material. Test III was remarkable for the consistent negative results obtained during a period of 11 days. This work is of value in that it indicates that there was little or no contamination of infusions or punctures from outside sources.

Tests III and IV were both made with infusions obtained from a badly infected upland field of Tip cane. During the period of the first of these, rain was of daily occurrence. The soil was kept wet during the entire period of Test III, which may have influenced the results. Test IV, with soil from the same field as Test III, yielded positive results during the entire period of the test. In this test positive results were obtained during a period of 24 days, indicating that the organism of red-stripe disease may live in the field soils as long as 24 days. Throughout this test, the number of positive infections was consistently low, with one exception. This may have been due to the slope of the land on which the original cane grew. These upland fields were at an elevation of 1,500 feet or more, where the disease is more serious and where daily showers are of regular occurrence during part of the growing season. The soil from which the infusions for Test V were made, was garden soil at Hawi, Hawaii, at an elevation of 400 feet. It was well watered during the test. There was no possibility of re-infection of the soil throughout the period of this latter test. The fairly consistent results obtained in this and other tests may be seen in Table II. The results obtained in Test V indicate that the organism may live in the soil as long as 32 days and still be capable of producing infection of susceptible cane leaves. The results in Test V would no doubt have been much higher had there been sufficient soil moisture present at all times where the host plants were growing. In Tests III, IV and V there appeared to be a gradual diminution of the numbers of the red-stripe bacteria in the field soils tested.

Table II

Summarized Table of Results on Five Tests for Soil Persistence of the Causal Organism of Red-Stripe Disease

No. of days since start of experiment	TEST NO. I April 30-May 17		TEST NO. II June 24-July 9		TEST NO. III July 21-August 1		TEST NO. IV August 5-August 28		TEST NO. V September 1-October 3	
	Number punctures made	Number inoculations positive	Number punctures made	Number inoculations positive	Number punctures made	Number inoculations positive	Number punctures made	Number inoculations positive	Number punctures made	Number inoculations positive
0	125	7	300	10	300	0	300	4	300	14
1	125	6	300	17	300	2	300	4	300	10
2	125	1	300	2	300	0	300	2	300	21
3	125	1	300	6	300	0	300	3	300	2
4	...	..	300	1	300	0	300	24	300	2
5	125	0	...	..	300	0	...	..	...	..
6	...	..	300	0	...	..	...	..	...	..
7	125	0	...	..	300	0	...	..	300	7
8	...	..	...	..	...	..	300	9	...	..
9	125	0	300	0	300	0	...	..	...	..
11	...	..	...	..	300	0	300	2	300	2
12	125	0	...	..	...	..	...	..	...	..
13	...	..	300	5	...	..	...	..	...	..
14	125	10	...	..	...	..	300	2	300	4
15	...	..	300	9	...	..	...	..	...	..
16	...	..	...	..	...	..	300	4	...	..
17	125	21	...	..	...	..	...	..	...	..
19	...	..	...	..	...	..	300	2	...	..
21	...	..	...	..	...	..	300	(2 stomatal)	300	0
24	...	..	...	..	...	..	300	3	300	1
28	...	..	...	..	...	..	...	..	300	0
30	...	..	...	..	...	..	...	..	300	3
32	...	..	...	..	...	..	...	..	300	11
Totals.....	1250	46	2700	50	2700	2	3600	59	3900	77

Part III

The Occurrence or Absence of Red-Stripe Infection on Young Plants of the Tip Variety from Healthy Seed Pieces Planted in Sterilized and Unsterilized Kohala Soil, Inoculated With the Red-Stripe Bacteria

This experiment was planned to corroborate, if possible, the results of the two previous experiments on the persistence of the organism in tubed soil and in field soil. It was planned so that field conditions could be simulated as much as possible. All the infections found and tabulated in this experiment are those which developed from contact of the young, newly formed plants pushing through this soil.

The soil used in this experiment was taken in duplicate from fallow cane land, on Hawi Plantation at Hawi, Hawaii. One lot was sterilized in shallow flats for 3 hours at 15 pounds pressure on each of 3 successive days. When completed, the soil was placed in 20 empty kerosene cans, each half filled. The cans were wrapped in muslin, with the covers replaced, and again sterilized. A duplicate lot of soil was taken, placed in twenty kerosene cans, each half filled. This duplicate lot was not sterilized. This soil is known as the "Unsterilized soil" used in this test. The kerosene cans in both the sterilized and unsterilized series were perforated on the bottoms for drainage.

The sterilized-soil cans were set up in the garden in a row to windward of the unsterilized soil cans. Two 400 cc. infusions of the red-stripe organism were made to a milky consistency from pure cultures on sucrose agar, using condensation water for the infusions. Twenty cubic centimeters of these infusions were added to the soil in both the sterilized and unsterilized soil cans. The soil was then stirred thoroughly with a sterile trowel. The sterilized soil was inoculated first, then the unsterilized soil. The covers were replaced on the sterilized soil cans and the unsterilized soil was covered with burlap bags to prevent excessive evaporation. There was no infected cane to windward of the site of this experiment, therefore air-borne infection was negligible. The covers were not removed from the cans until they were ready to be planted.

Seed cane was planted in these soil cans at different time intervals. Seed of Yellow Tip cane with strong vigorous healthy eyes was obtained from a healthy field and washed free from dust in tap water. Two seed pieces of at least three eyes each were planted side by side in each can. Usually the seed pieces were as long as the can could accommodate. Each time seed was planted it was handled in the same manner and planted within 2 hours of the time it was cut. Five cans from the sterilized and five cans from the unsterilized series were planted at each interval. Lot numbers were given these lots of 5 cans each. The sterilized lots of soil bore the lot number with the letter "A" following, for identification. The same lot number was given the lot of unsterilized soil planted the same day, but without the letter following.

Lot V consisted of 5 kerosene cans half filled with identical soil, unsterilized and not inoculated. In each of these cans on September 20, two healthy seed pieces of Yellow Tip seed, from the same source as the other seed, were planted.

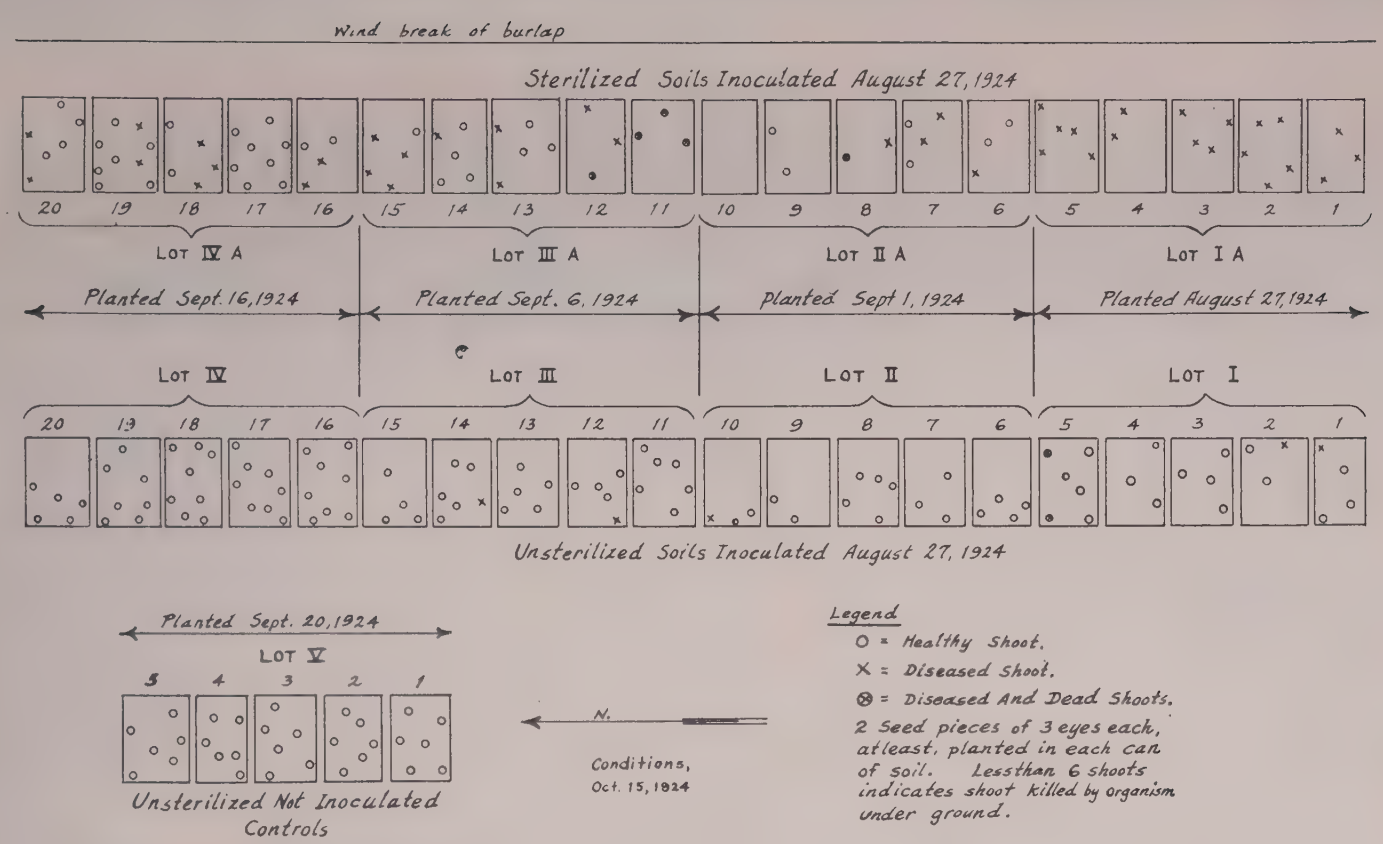

Fig. 12. A chart showing the positions of the cans used as soil containers in the test of sterilized and unsterilized soil. The dates of planting each lot and the number and condition of each stalk at the conclusion of the test are also shown.

Each seed piece consisted of three healthy eyes. This lot was planted to prove that the infection at that time developing on the cane planted in the inoculated soil was due to the bacteria in the soil and not to air-borne bacteria. All water used for irrigation of the soil used in this experiment was sterile condensation water from local sugar mills. It became necessary to add sodium nitrate to all soils when the plants showed poor growth following germination.

The accompanying chart, Fig. 12, shows the position of plantings, the number of stalks resulting in plantings, and the presence of diseased and dead stalks.

Results of Tests:

In the sterilized soil the results of this test supported the determinations made on tubed soil. There was much more direct infection of shoots in the sterilized soil than in the unsterilized soil. In the sterilized soil 48.7 per cent of the shoots were badly infected, most of them showing infection at the time the shoots pushed above the surface of the soil. In the unsterilized soil only 7.7 per cent of the shoots which appeared became infected. In the controls there was no infection present during the entire test.

In the matter of germination of the eyes, the seed pieces in the controls developed a healthy shoot from each eye. These shoots remained healthy through-

Fig. 13. At the left is a stool of cane growing in unsterilized soil to which an infusion of the red-stripe organism was added. At the right is a stool of cane growing in similar soil, sterilized, to which an identical amount of the same infusion had been added. These stools are typical of the plants in both series of pots and show the effect the red-stripe organism had in lessening germination and growth of roots and shoots.

out the test. There was markedly less germination in the sterilized and unsterilized soils that had been inoculated. In many cases seed pieces contained four eyes from which only one shoot appeared. In all cases at least six eyes were planted in each soil can. Where a lesser number than six shoots appear in Fig. 12 the shortage may, in part at least, be attributed to direct infection and inhibition of the germinating eye. When the experiment was concluded a careful examination of the seed pieces proved that germination had started in many cases, but that severe infection had prevented many shoots from reaching the surface of the soil. The photograph reproduced in Fig. 13 shows the much greater root development and aerial growth of the plants in unsterilized soil, where the red-stripe organism was minimized by the normal soil organisms, than of the plants in sterilized soil. This difference was characteristic of all pots in the two series.

There were only 82 sprouts that appeared in the sterilized soil. Ninety-nine plants appeared in the unsterilized soil. There should have been approximately 240 shoots in each of the two preparations of soil if germination had been equal to that obtained in the controls. The control lot, having exactly 30 eyes planted, produced just 30 healthy shoots. In the sterilized soil the average number of days after planting until the first appearance of disease was 19.5 days. In the unsterilized soils the average number of days until the first appearance of diseased stalks was 15 days. In the first three plantings in sterilized soil the diseased stalks first appeared in an average of 16.5 days for the three lots. The last planting of cane in the sterilized soil was very late in becoming infected but there was no doubt that this infection developed from direct contact with the soil. Infection occurred in sterilized soil up to 49 days after the soil had been inoculated.

The most important result of this experiment is the evidence obtained showing that the causal organism does not increase in numbers in unsterilized soils that have been inoculated with the organism. This point is shown in the diminution of infected plants in the late-planted pots. Each successive lot of unsterilized soil had less infected plants and better germination until the last, which had no infected plants at the conclusion of the experiment. The experiment shows that the causal organism does not remain in the soil indefinitely, but gradually disappears. Since in sterilized soil the reduction does not take place as rapidly, the reduced activity in unsterilized soil can presumably be ascribed to the presence and activity of the normal soil organisms in such soil. The following table shows in detail the most important facts which were determined in this experiment:

TABLE III

Results of Planting Healthy Cuttings of Yellow Tip Variety at Various Time Intervals in Series of Pots of Unsterilized Soil Inoculated With an Infusion of the Red-Stripe Bacteria, and in a Similar Series of Pots of Sterilized Soil Inoculated With the Same Bacterial Infusion. Each Lot Consisted of 5 Pots. All Soil Pots Except Controls Were Inoculated August 24, 1924.

Lot number	Soil Treatment	Date of planting	Interval between soil inoculation and planting of seed (Days)	Period in days between soil inoculation and appearance of stalks	Total stalks in each lot of 5 cans	No. of diseased stalks in each lot	Percentage of diseased stalks	No. stalks killed by organism
I A	Sterilized	Aug. 27	0	8	19	18	94.7	0
I	Unsterilized	Aug. 27	0	7	20	4	20.0	2
II A	Sterilized	Sept. 1	5	14	11	5	45.4	1
II	Unsterilized	Sept. 1	5	14	17	1	5.8	0
III A	Sterilized	Sept. 6	10	19	21	8	38.0	4
III	Unsterilized	Sept. 6	10	19	26	2	7.7	0
IV A	Sterilized	Sept. 16	20	28	31	9	29.0	0
IV	Unsterilized	Sept. 16	20	28	36	0	0	0
V	Control—not inoculated	Sept. 20	,,	,,	30	0	0	0

The writer wishes to acknowledge the splendid cooperation of the plantation managers of the Kohala district in the course of the field tests on red-stripe disease reported here and elsewhere. The many helpful suggestions and contributions to the work made by W. C. Jennings, of this Station, are also highly appreciated.

The writer desires to thank H. Atherton Lee and J. P. Martin, of the laboratory in Honolulu, both of whom contributed a great deal toward the success of the problem.

Conclusions

In discussing the activities of the causal organism of red-stripe disease in Kohala soils, it has been very clearly shown that the organism may live in these soils in infected fields for periods of at least 32 days and that during this time there is a definite reduction in numbers of these bacteria present in the soil. This fact naturally leads to the conclusion that the mud carried on implements, shoes or vehicles from badly infected cane fields would be a source of infection for susceptible canes in other districts in cases of immediate movement to such districts under favorable weather conditions. It should be clearly pointed out that under field conditions, the upland infected cane fields of Kohala are, during the growing period of the plants, becoming almost daily re-infected with very active bacteria from the diseased leaves through the agency of daily winds and showers. It has been clearly established in these experiments that the bacteria are washed into the soil by rains from the leaves of diseased cane plants.

Although we have proved that the causal organism lives in the soil over periods of 32 days it does not apparently multiply there. The gradual diminution of positive results toward the close of both the tests on tubed soil and field soil most certainly indicated this point.

The question of the passive existence of the organism in the soil is also well answered. In the tubed sterilized soil and in the sterilized soil in which cane was grown a definite reduction in numbers was recorded. These facts all point to gradual loss of activity of the organism as well as reduction in numbers present during periods of time approximating 40 days. In the tubed sterilized soil the reduction in numbers was not due to competition of other organisms. The reduction in numbers of bacteria in the sterilized soil in which cane was grown, as interpreted by the number of infected shoots which appeared, may have been partially due to competition with air-borne organisms introduced at and after the time of planting. The results obtained from this experiment, in which the cane was grown in inoculated soils, very conclusively confirms the results obtained in the tests of soil persistence made through artificial inoculations of cane leaves. The persistence of the organism in the sterilized soil, as indicated by the natural infection of the roots and stalks grown in this soil, even of the latest plantings, indicates the ability of the causal organism of red-stripe disease to persist in sterilized soil over long periods of time. This test also proves that rains and adverse weather conditions did not appreciably reduce the period of activity in these exposed soils. The clean-cut reduction in numbers of infections on the later plantings in the unsterilized field soils which had been inoculated concurrently

with the sterilized soil, points to the gradual diminution of numbers of bacteria, as was shown by artificial inoculation, to result in the tubed unsterilized soils. The results obtained in this test substantiate the findings in the other tests most definitely and assured the dependability of the methods used.

The steady reduction of numbers in unsterilized field soils and in unsterilized tubed field soils is, however, much more rapid than in the sterilized soils, and this increased rate of reduction in numbers may be attributed, in part at least, to competition with native soil organisms, such as beneficial soil bacteria, fungi, algae, protozoa and other soil-inhabiting plants and animals.

It may be assumed from these experiments that the widespread infection of ratoon fields of Tip cane may in part be due to the presence of bacteria in the soil washed down by rains immediately previous to harvesting the previous crop of cane. In the process of cultivation these organisms would be brought to the surface and spread by winds and splashing rains to the sprouting cane. A few such infections scattered over a large field would thus become centers for wind distribution within a few days and insure wholesale distribution in a very short time. With plant cane, however, the very widespread occurrence of infection within several weeks from planting time can hardly be ascribed to soil-inhabiting bacteria, since the preparation of the field previous to planting usually occupies a much longer period of time than has been determined for the activity of the causal organism of red-stripe disease in these soils. Such general infections of entire fields of plant cane must, therefore, be due to wind-borne bacteria from infected fields of Tip cane to windward of the young cane. It is not entirely improbable that some of the infection also may come from the field soil, in cases where the previous plantings were of the Tip variety and heavily infected with the disease.

A Comparison of Red-Stripe Disease With Bacterial Diseases of Sugar Cane and Other Grasses

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By H. Atherton Lee, Helen A. Purdy, Clyde C. Barnum and J. P. Martin

The outbreak of red-stripe disease of sugar cane in the Kohala district, rather far from Honolulu, and well isolated by natural conditions, has created an interesting question as to the way in which the disease developed there. The first probability which seemed suggestive, was the spread of the disease to the susceptible Tip canes, from native varieties growing in small dooryard plantings which are common on Hawaii. Search for the disease in such small dooryard plantings showed in no case, however, any traces of the disease, although some of these native Hawaiian varieties have been shown to be very susceptible in plantings exposed to infection.

The next possibility which seemed suggestive was the introduction of a disease on a related grass, which upon becoming established in Kohala spread on to the susceptible Tip varieties. This paper discusses this possibility, and in so doing deals with the relationship of red-stripe of sugar cane with other bacterial cane diseases and with bacterial diseases of grasses other than cane.

Other Bacterial Diseases of Sugar Cane

Two diseases of sugar cane have been known previously for which bacterial species have been established as the causal agents. Gumming disease of sugar cane, which was first described by Cobb(1) in Australia, was first definitely shown to be due to bacteria by Erwin F. Smith (16). It is clearly very different from red-stripe disease of Tip canes, since it is not only a disease of leaves but also when an affected cane stalk is cut a yellow gummy ooze is produced which is distinctive. The fact that it affects such varieties as H 109, Rose Bamboo and Lahaina, which are not affected by red stripe, also indicates clearly that it is a different trouble.

Leaf scald is another bacterial disease of sugar cane which occurs in Java, the Philippines, Australia and Fiji. It affects H 109 and Lahaina varieties, and in addition exhibits differences from red-stripe disease in leaf symptoms and is, therefore, quite distinct. It is, moreover, caused by a rod-shaped organism much smaller than the bacterial species causing red stripe of the Tip varieties. The etiology of leaf scald has been established by Miss Wilbrink(26) in Java, and North in Australia, independently. North's work has not yet been published.

Other than these diseases, we know of no bacterial diseases of cane which have been definitely established. Earle(3) has published a short note of a trouble in Porto Rico which caused short, purplish-brown streaks about one-eighth to one-fourth inch long. The spots became very numerous and the leaves took on a seared appearance. Earle suggested that the symptoms were much like those of some of the well-known bacterial leaf spots. The trouble occurred on the variety Demerara 109 and other varieties, although these other varieties were not mentioned. The description of these symptoms indicates that they are quite unlike the long, red stripes, which occur on Tip canes due to red-stripe disease, although this difference could be due to the different reaction of the varieties affected in Porto Rico. Until the etiology of this Porto Rican disease is known there is not sufficient evidence to regard it as the same as the red-stripe disease of Tip canes in Kohala.

Tryon(24) has described a disease of sugar cane in Australia called top rot, the principal leaf symptoms of which are a yellowing, or in some cases browning, of the leaves. In some stages he mentions, however, that narrow, reddish streaks are formed, which has raised the question as to its possible identity with the red-stripe discussed in this paper. Tryon, however, associated with the trouble a fungus in the roots of affected cane; moreover, the varieties Rose Bamboo, Striped Mexican and Lahaina are affected, and since these varieties are not affected with the red-stripe disease found here, the top rot of Australia would seem to be distinct from red-stripe disease.

Polvillo, a disease of sugar cane in the Argentine, was first described by Spegazzini (22, 23) and a short account of it has been given by Erwin Smith (17). A more recent account, by G. L. Fawcett (6), mentions a reddish stained color upon the bud, and stripes of the same color on the leaves. Bacteria are suspected of being the causal agents, but their causal relationship has not been clearly established. The varieties P. O. J. 213 and P. O. J. 234 are the susceptible canes, according to Fawcett. There are no data available as to the susceptibility of these varieties to red stripe here in these Islands. Erwin Smith, however, quotes Spegazzini to the effect that Cana Rayada is a susceptible variety. If this variety be taken to be Striped Mexican, as is quite probable, then the trouble would not be the red-stripe disease of these Islands, since Striped Mexican has not been affected with red stripe here. Spegazzini also described the cane stalk as affected, sometimes its entire length down to the roots, which would differ from the red-stripe disease of Kohala, which does not affect the cane stalk except in a very few cases at the topmost internodes. Polvillo is, therefore, apparently different from red-stripe disease.

Bacterial Diseases of Grasses Other Than Sugar Cane

In considering the possible identity of diseases of grasses other than sugar cane with red-stripe disease, differences in symptoms are of a questionable value in some cases, due to possible differences in reaction of the same organism on different host plants. For this reason a comparison of the characters of the causal organisms of these other grass troubles is of more value than a comparison of symptoms or parts of the plant affected.

Of the diseases on related grasses, a disease of sweet corn, *Zea mays,* is known in the United States, sometimes called Stewart's disease. Erwin F. Smith (15) established the causal relationship of a bacterial rod-shaped organism forming yellow colonies on agar. The organism is obviously different from the whitish organism of red stripe of sugar cane.

Rosen (12) has established the causal relationship of a bacterial species to a disease of foxtail, *Chaetochloa lutescens.* The causal organism of this disease is rod-shaped, with but one polar flagellum, has capsules, is strictly aerobic, has strong diastasic action, and has a thermal death point between 41° and 43° C. The red-stripe disease organism of sugar cane is a bacterial rod with one to several polar flagella, has no capsules, is a facultative anaerobe, has very slight, almost no diastasic action, and has a thermal death point of 52° C. There is clearly a difference between the organisms causing these two diseases.

O'Gara (9) has established the causal relationship of a bacterial organism to a disease of western wheat grass, *Agropyron smithii,* which occurs in Utah. This organism forms yellow colonies on agar, which seems to distinguish it from the whitish organism of red-stripe disease of sugar cane.

Erwin Smith (18) also gives a brief account of a paper by Rathay (10) which describes a disease of orchard grass, *Dactylis glomerata,* which occurs in central Europe. The bacterial organism associated with the trouble forms colonies of a lemon-yellow color on potato cultures, while, as previously mentioned, the sugar cane red-stripe organism is whitish on such media. The characters of the

lesions of the disease of orchard grass are of an entirely different type from those of red stripe of sugar cane, and the two diseases are quite certainly distinct.

Halo blight of oats, *Avena sativum,* is a disease occurring in the central and western United States. The causal agent is a rod-shaped bacterial organism with capsules, forming white colonies on agar; nitrates are not reduced, and the thermal death point is between 47 and 48° C. The pathogenicity and essential characters of the organism were determined by Miss Charlotte Elliott(4). The quickly notable differences between this organism and that of red stripe of sugar cane are in the reduction of nitrates, which does not occur for the oat organism but is rapid in the red-stripe organism. The thermal death point of the oat organism is between 48 and 50° C., and is between 52 and 53° C. for the red-stripe organism.

Bacterial blight of barley, *Hordeum vulgare,* occurs in the central and western United States, and is caused by a bacterial rod-shaped organism as established by Jones, Johnson and Reddy (7). The organism forms wax-yellow colored colonies on agar and does not reduce nitrates. Since the causal organism of red stripe of sugar cane forms white colonies on agar and reduces nitrates readily, the organisms are clearly distinct.

Black chaff of wheat, *Triticum* spp., which occurs throughout the middle western United States is caused by a bacterial rod which is distinguishable from the organism of bacterial blight of barley only in its ability to attack the leaves, culms and glumes of wheat plants. Since it forms yellow colonies on agar and does not reduce nitrates there is no possibility of its connection with the organism of red stripe of sugar cane. The work upon this disease was by Erwin Smith, L. R. Jones and C. S. Reddy (21).

A disease of rye, *Secale cereale,* is caused by a bacterial organism identical morphologically and in culture with the organism of barley blight and black chaff of wheat. It differs only from these organisms in that it is pathogenic to rye only and does not affect wheat or barley. The research upon this disease has been by Reddy, Godkin and Johnson (11). Since it forms yellow colonies on culture media and does not reduce nitrates, it is clearly distinct from the whitish nitrate-reducing organism of red-stripe disease of sugar cane.

Another disease of wheat known as basal glume rot has been reported by Miss Lucia McCulloch(8) from the northern United States and western Canada. Miss McCulloch has established the causal relationship to the disease of a rod-shaped bacterium with capsules, forming white colonies on agar. The formation of capsules and the absence of nitrate reduction serve to distinguish this organism from the organism of red-stripe disease of sugar cane.

Erwin F. Smith(19) mentions bacterial diseases on grass species of the genera *Bromus, Phleum,* and *Poa,* for which we have not been able to find the literature in Hawaii. It seems probable that Dr. Smith has worked with these diseases but has not as yet had the opportunity to publish upon them.

Rosen (12), whose publication has been very valuable to us in reviewing bacterial diseases of grasses, also lists a publication by Voglino(25) which is not available to us, describing a disease of rice in Italy. Since the bacterial rods of this causal organism are rather large, as Rosen points out, the disease apparently has no connection with the red stripe of sugar cane, the causal organism of which has much smaller bacterial rods.

Cross Inoculations With Red-Stripe Bacteria on Other Grasses

In addition to a comparison of these known diseases of grasses, inoculations have been made with the bacteria of red stripe of sugar cane, on a number of related grasses which occur naturally in the Kohala district to determine other possible host plants. Sweet corn and field corn, *Zea mays;* crab grass, *Syntherisma sanguinalis;* Guinea grass, *Panicum maximum;* goose grass, *Eleusine indica;* Wilder grass, *Chloris radiata;* Dallas grass, *Paspalum dilatatum;* Johnson grass, *Holcus halepensis;* Hilo grass, *Paspalum conjugatum;* elephant grass, *Pennisetum purpureum;* red top, *Tricholaena rosea;* and foxtail, *Chaetochloa verticillata,* have been tested. Inoculations were made with the red-stripe organism direct from agar-slant cultures, with needle punctures, and the conditions maintained most favorable for infection. Inoculations upon all of these grasses resulted negatively with the exception of the inoculations on sweet corn, field corn and Johnson grass.

In the case of Johnson grass, small red margins appeared around the inoculation punctures which did not appear around the control punctures. Nothing resembling stripes appeared, however. In the case of the sweet corn, the leaf tissues turned yellow and watery surrounding the inoculations. From 200 inoculations made on healthy leaves of sweet corn, 168 developed watery yellow areas, which indicated a definite necrosis of the tissues resulting from the presence of the organism causing red-stripe of sugar cane. Control punctures did not develop these watery areas in any case. No stripes were formed which resembled the red stripe of sugar cane. With field corn the results were almost identical. The controls did not produce any lesions, while the inoculations yielded 72 per cent positive results. The watery lesions produced on field corn were much larger than those on sweet corn, but in no case did any red color appear. All tests were repeated with the same results; all the grasses gave entirely negative results, with the exception of the Johnson grass, and on this grass the lesions were not stripes but discolorations such as resulted in the first tests. The inoculation data and results are on record in the laboratory files and are available to an investigator who may be interested in the details. These results serve to indicate that none of our common field grasses occurring as weeds are of importance as sources for infection of cane with red stripe. Moreover, the results add to the evidence indicating that the red-stripe disease of sugar cane is distinct from Rosen's disease of foxtail and Stewart's disease of corn.

Sorghum Blight

A disease occurs in the middle-western and western United States which is known as sorghum blight. Erwin Smith and Miss Florence Hedges (20), in describing the lesions of the disease, use the words "elongating red-brown blotches;" they established the causal relationship of a rod-shaped bacterial organism with this disease. Erwin Smith(14) states, "It is non-sporiferous, polar flagellate (1 to 3), and white on culture media, forming small circular colonies on agar-poured plates. It is aerobic, non-liquifying, non-reducing (nitrates)." Leaves, leaf sheaths and roots are affected. Erwin Smith(13) has also published a photograph of such lesions of the disease.

Because the organism of this disease did not reduce nitrates, we at first were not greatly concerned with it, but subsequently found a small planting of sorghum on Oahu affected with this trouble. It had not been previously reported from these islands. Points increasing our suspicion of this trouble were the facts that forage grasses had recently been introduced from the mainland to an area close to this sorghum, and that forage grasses had been shipped from this vicinity to Kohala. No cane of the Tip varieties was growing within miles of this area, which we considered might explain the absence of the disease from cane on the island of Oahu.

In order to carefully prove or disprove the identity of the red striping of sorghum with the red stripe of sugar cane, sorghum, *Holcus sorghum*, was grown in the Kohala district and inoculated with the bacterial organism of red stripe of sugar cane. Inoculations were made with needle punctures, introducing the inoculum from agar streaks into the punctures. Inoculated leaves were moistened with water, wrapped in paraffin paper to maintain the moisture on the leaves, and then wrapped in opaque paper to protect the inoculations from the sun. Control inoculations were made identically, but without the bacteria of red stripe of sugar cane. Ten days after inoculation small red markings and, in a few cases, narrow red streaks resulted at the punctures with the sugar cane organism. The control punctures remained entirely normal.

TABLE I

Results of Inoculation With the Organism of Red Stripe of Sugar Cane from Agar Slants on Sorghum Plants. Inoculations made July 19, 1924; Results Recorded July 29.

Stalk No.	Number of positive results	Percentage punctures positive	Character of streaks
1	4	40	Narrow red margins around punctures.
2	7	70	Several narrow red streaks ¼ to ½ inch long.
3	1	10	Red margin around puncture.
4	plant lost	..	.....
5	2	20	Narrow red streaks less than ½ inch long.
6	6	60	Several narrow red steaks, several 1 inch long.
7	1	10	Narrow streak 1 inch long.
8	1	10	Narrow streak less than 1 inch long.
9	2	20	Red margins around punctures.
10	7	70	Several narrow streaks 1 to 2 inches long.

The experiment was repeated with almost identical results. In this test, there were several inoculations which resulted in narrow streaks 1 to 3 inches long, while the controls remained entirely normal. Another repetition of the test, in which twenty plants were inoculated and ten controls maintained, showed no red streaks, but red margins developed around the inoculations, while control punctures remained normal. A fourth series of tests was made in which ten plants were inoculated with ten plants as controls; in this series the punctures with the inoculum also yielded a number of narrow red streaks from ⅛ to 3 inches long, while control punctures remained normal. In all of these tests we were able to get red streaks of a very suspicious nature, but could not secure the broad red

streaks, and long streaks of the naturally occurring sorghum blight. A last test, with ten plants punctured with the inoculum and ten controls gave no streaks but simply reddened margins around the inoculated punctures.

Inoculations were, therefore, undertaken on healthy Tip canes, using crushed fresh tissues from new lesions of sorghum blight; the inoculum was introduced into the cane leaves with needle punctures. Controls were run simultaneously with needle punctures and no inoculum. Ten inoculated plants were tested and ten plants used for the control punctures. All inoculated leaves were moistened, wrapped in paraffin paper to maintain the moisture and then wrapped with opaque paper to protect the inoculations from sunlight. At the end of fifteen days all punctures with the inoculum were entirely negative and comparable to the controls in their color changes or extent of healing.

TABLE II

Results of Inoculations With Crushed Material of Young New Lesions of Sorghum Blight on Leaves of Sugar Cane of the Yellow Tip Variety

Stalk No.	Nature of inoculum	Percentage of positive results 15 days after inoculation
1	Tap water	0
2	" "	0
3	" "	0
4	" "	0
5	" "	0
6	" "	0
7	" "	0
8	" "	0
9	" "	0
10	Sorghum-blight tissues	0
11	" " "	0
12	" " "	0
13	" " "	0
14	" " "	0
15	" " "	0
16	" " "	0
17	" " "	0
18	" " "	0
19	" " "	0
20	" " "	0

The experiment was repeated with ten inoculated plants and ten control plants with identical results; no red stripe resulted on the Tip cane from the crushed tissues of the sorghum-blight tissues.

To corroborate this, the area of sorghum showing the blight was interplanted with healthy seed of Tip cane varieties, with the idea that the abundant infection on the sorghum might spread by natural means to the young Tip cane. No red stripe of the sugar cane resulted after six months. The sorghum and Tip canes were then sprinkled artificially for several hours daily to make the conditions most favorable for red-stripe infection, but none resulted.

To summarize the data, the results showed that the organism of red stripe of sugar cane would produce small, narrow lesions on sorghum, which were not, however, comparable to sorghum-blight lesions, but that sorghum-blight material inoculated on Tip canes was entirely non-pathogenic to the sugar cane.

It would, therefore, seem rather clearly established, that the blight of sorghum is distinct from red stripe of sugar cane.

Bacterial Stripe Disease of Proso Millet

A disease of proso millet has been described by Miss Charlotte Elliott(5) which occurs in South Dakota and Wisconsin, and for which Miss Elliott has established the causal relationship of a bacterial species, *Bacterium panici*. In her publication a colored plate shows long, red stripes, typical of the disease on millet, which are very suggestive of the red-stripe disease on sugar cane. The causal organism of the millet disease, as described by Miss Elliott, is a motile rod with polar flagella, white on culture media, with capsules; nitrates are reduced and diastasic action is moderate. These characters are in rather close agreement with those of the red-stripe organism of sugar cane, with the exception that the sugar cane organism has no capsules and has a very faint diastasic action on starch media.

The similarity was so suggestive, however, that inoculations of the sugar cane organism were undertaken on proso millets. Miss Elliott was kind enough to send seed of the Early Fortune millet with which she had readily obtained positive results with her organism. Seeds were also obtained of German millet, Russian millet, Hungarian millet, Japanese millet, Hogar millet, White Siberian millet, Black Voronezh millet and White Ural millet.

Using the same methods described for the inoculation of sorghum, these millet varieties were inoculated with the sugar-cane organism, with needle punctures, ten plants of each variety to a series. All plants remained entirely negative. More plants of each variety were grown from seed and again inoculated, with definitely negative results. Since Miss Elliott's inoculations on Early Fortune millet were secured by stomatal infections and with no punctures, this method was also tried, with definitely negative results. The results with the Early Fortune millet only, are tabulated here in order to minimize page space.

Table III

Results of Inoculations With the Organism of Red Stripe of Sugar Cane from Agar Slants on Plants of Early Fortune Millet. Inoculations Made October 4; Results Recorded October 14, 1924

Stalk Number	1	2	3	4	5	6	7	8	9	10
Percentage of positive results	0	0	0	0	0	0	0	0	0	0

It did not seem a safe procedure to introduce the proso-millet organism into the Hawaiian Islands, but at Dr. Erwin Smith's request, healthy cuttings of the Yellow Tip variety of sugar cane were sent to his laboratory in Washington, where Miss Elliott inoculated them with the proso-millet organism. Miss Elliott has written that her inoculations with the proso-millet organism on the Yellow Tip sugar cane in Washington have given negative results.

Discussion

It seems rather clearly established that the organism of red stripe of sugar cane is distinct from the organisms of sorghum blight and bacterial-stripe disease of proso millets. Red-stripe disease is also clearly distinct from Cobb's gumming disease and leaf scald. It is possible that Earle's disease of the variety Demerara 109 of sugar cane in Porto Rico is identical, yielding different symptoms on such a different variety, but it does not seem probable. The disease, polvillo, in the Argentine, might be identical also, but judging from the varieties of cane affected by it, the identity does not seem probable. An unpublished illustration of a pathological condition is available from D. S. North, in Australia, showing long, red stripes on sugar cane, and the identity of this trouble with red-stripe disease of Kohala is a matter which could be advantageously investigated. As yet, however, there is no evidence sufficiently substantial to base any explanation of the origin of red-stripe disease in the Kohala district.

The writers of this paper would have preferred to refer this bacterial organism to some previously described species if it were possible, but it seems rather clear that this pathogene causing red-stripe disease of sugar cane is distinct from other previously known pathogenic organisms on sugar cane, and from the pathogenic organisms of other grasses as well.

If the arrangement of classifications of the Committee of the Society of American Bacteriologists(2) is followed, this organism, being pathogenic to plants and with polar flagella, is to be referred to the genus *Phytomonas*. In order to facilitate further discussions of this organism the binomial *Phytomonas rubrilineans* is suggested, meaning, the *Phytomonas* making red stripes. The technical description is as follows:

Phytomonas rubrilineans sp. nov.

Baculis brevibus cylindricis, apicibus rotundatis, singulis aut saepe geminis, vulgo 1.6 x 0.7 μ; motilibus, flagellis 1-3 polaribus; sporis capsulis que nondum visi; methodo Grami non coloratis; coloniis in agar-agar orbicularibus, nitentibus, convexis, luteo-pallidis, prope lacteibus; gelatinis lente liquefacientibus, lactem litmus celeriter decolorantibus; nitrum reducentibus, ammoniam producentibus; indol non evolvatis; in mediis saccharatis gas non evolvatis sed in presentia sacchari uvae acidum producentibus; facultative anaerobicis. In foliis vivis *Sacchari officinari* rubidas lineas efficiens.

Summary

(1) A review of the known diseases of sugar cane, of which bacterial species have been well established as the causal agents, shows no similarity of any of these species with the organism of red-stripe disease of sugar cane of the Kohala district.

(2) There are three diseases of sugar cane, the polvillo in the Argentine, a red leaf blemish in Porto Rico, and a top rot in Australia, for any one of which there is a remote possibility of identity to the red stripe of sugar cane in Kohala, but the identity in no case seems probable. The causal agents for these three diseases have not yet been determined, and therefore no comparison is possible with the causal organism of red-stripe disease.

(3) Of eleven bacterial diseases of grasses other than sugar cane, which have been studied and whose causal relationships have been well established, nine have been reviewed in this paper, and because of very obvious differences in the characters of the causal bacteria, it can be definitely said that none of these nine diseases have any connection with the red-stripe disease of sugar cane in Kohala.

(4) The two remaining diseases, sorghum blight and bacterial stripe disease of proso millet, have been viewed with suspicion as possibly identical with red stripe of sugar cane. Cross inoculations of the sugar-cane red-stripe organism on sorghum have yielded narrow, short, red stripes which, although suspicious, could not be considered identical with the long, broad blotches of sorghum blight. Inoculations of young, fresh, crushed, diseased tissues of sorghum blight tissues into leaves of Tip canes have been entirely negative. It seems reasonable to conclude that sorghum blight is a disease distinct from red stripe of sugar cane.

(5) Cross inoculations with the sugar-cane red-stripe organism on young plants of proso millet have repeatedly resulted negatively. Reports from Washington indicate that the proso-millet organism inoculated on Tip canes in quarantine greenhouses in Washington have also resulted negatively. It seems reasonable to conclude that the red stripe of sugar cane is distinct from the bacterial stripe of proso millet.

(6) The red-stripe disease of sugar cane to the present time, therefore, cannot be connected with any of the known diseases of sugar cane or related grasses either in these Islands or other countries. There is no evidence whatsoever of the disease having been introduced on sugar cane or related grasses, and there has been developed, as yet, no suggestive evidence to indicate the origin of the disease in Kohala.

(7) The causal organism of red-stripe disease of sugar cane is a bacterial species, hitherto undescribed, and for convenience in further discussions it has been given the binomial, *Phytomonas rubrilineans* sp. nov., the *Phytomonas* forming red stripes.

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The Histology of Red-Stripe Disease

By H. Atherton Lee and D. M. Weller

A knowledge of the host tissues affected by a disease often leads to a better understanding of the methods of its dissemination, character and amount of injury, and in some cases may aid in selecting varieties resistant to the trouble. For this reason it seems desirable to record briefly the knowledge which has been obtained of the histology of red-stripe disease of sugar cane.

Methods Employed

Leaves of sugar cane, especially the older leaves where red-stripe disease is fully developed, have tough, rigid vascular bundles which cause paraffin sections to tear when cut transversely on a rotary microtome. Often the microtome knife is badly nicked by these tissues. The junior writer of this article, therefore, resorted to the use of a sliding microtome; the object was not imbedded in paraffin or other material, but was placed between pieces of elderberry pith. Sections eight micromillimeters in thickness were obtained by this method without difficulty. The sections were uniform in thickness and flat, as is evidenced by the photomicrographs. The studies reported here were from sections of leaves of the Yellow Tip and Striped Tip varieties, which are the only extensively grown varieties in these Islands which are to any extent susceptible.

To show the bacteria in the tissues, carbol fuchsin was first used, staining for but a second or two and decolorizing the host tissues to some extent subsequently with alcohol. It was found that the method picked out the lesions of the disease markedly; lesions would stain a reddish brown, while normal tissues would be unstained. With a counter-stain of light green, which was taken up by the normal tissues, distinct differentiation was obtained between healthy tissues and the lesions of the disease. Coleman and Bell stains were used.

Entrance of the Causal Organism in the Cane-leaf Tissues

Red stripe of sugar cane is primarily a leaf disease and, although infection occasionally runs down into the cane top, infection usually is initiated in the leaves. Studies of microtome sections of very young, naturally occurring leaf lesions have shown infection apparently spreading from the stomata. The carbol fuchsin in a number of sections has picked out the guard cells of the stomata and the parenchyma cells surrounding the sub-stomatal chamber, showing their pathological condition. In this way infection in many instances can be traced to the stomatal openings. Infection through one of the stomata is shown in the photomicrograph reproduced in Fig. 14. In sections of older lesions, however,

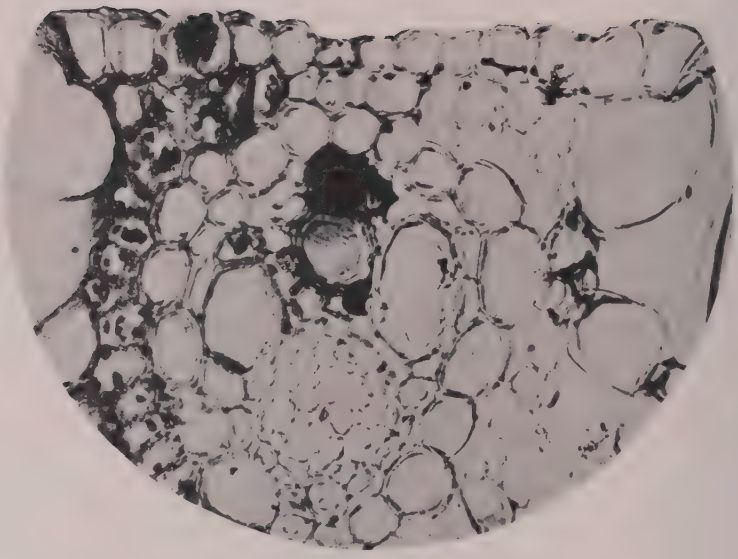

Fig. 14. This photomicrograph X 750 is from a slide stained with carbol fuchsin which has shown the infected chlorophyll-bearing parenchyma cells and infection spreading into the xylem. The way in which the stain has picked out the mass in the stomatal opening between the guard cells leads to the inference that infection took place in the case of this lesion, through this stomatal opening. In this section the sclerenchyma cells, starch sheath cells, and phloem are entirely unaffected although adjacent to the well infected parenchyma cells.

the point of infection is obscured by general infection of all tissues surrounding the stomata.

The inference that infection takes place through stomata is substantiated by the results of inoculations with infusions from cultures of the red-stripe bacteria. In experiments by Barnum, reported elsewhere, in which an infusion was applied with a small camel's-hair brush to the upper and lower leaf surfaces with and without needle punctures, it was shown that infection may take place through the stomata without wounds. From our studies of these sections it would appear as if entrance to the leaf tissues under natural conditions in the field was principally through the stomata, although where wounds occur infection may take place readily. Erwin Smith(1) has pointed out the same conclusions for sorghum blight, a disease of a similar character on sorghum.

The Tissues of the Leaf Affected

Our first studies were entirely on sections of young, new lesions, and in such sections the parenchyma cells were chiefly affected. The sclerenchyma cells, epidermal cells, motor cells, and the sheath cells which surround the vascular bundles were not observed to be affected. We had almost reached the conclusion that infection did not get into any of the parts of the vascular bundles, but ultimately found several xylem cells which showed infection. Subsequently, in

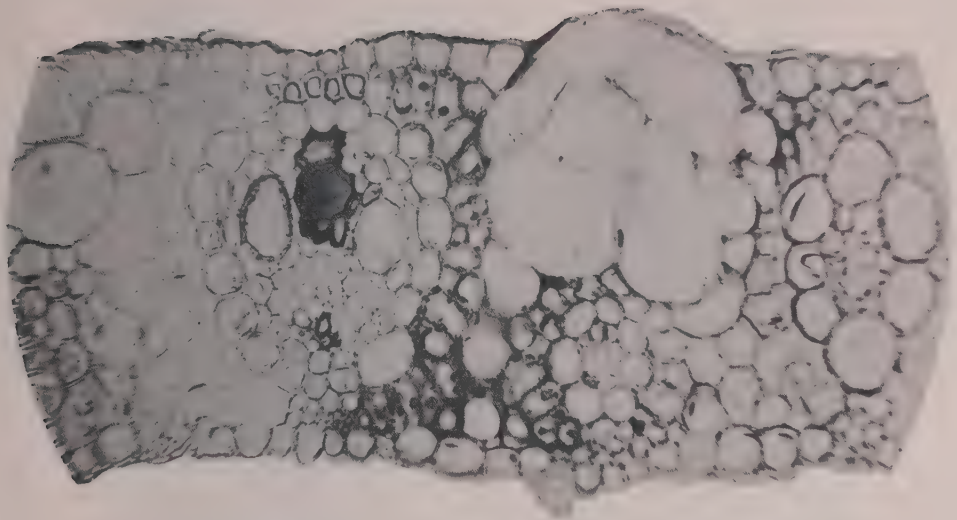

Fig. 15. This section of a new lesion, stained with carbol fuchsin, shows the infection in the chlorophyl-bearing cells of the parenchyma and in the xylem. The epidermal cells, sclerenchyma and sheath cells are unaffected, although one or two of the phloem cells seem to have been invaded. The large cells to the right of the vascular bundle are motor cells which we have observed to be but seldom affected. The photomicrograph is X375.

examining sections of old, well developed lesions, however, the xylem tissues were found to be very generally infected, and even in a few cases the phloem. Xylem infection is shown in Figs. 14, 15 and 16. A few epidermal cells also sometimes show infection. In old lesions, subsequently, the sclerenchyma cells, sheath cells, and motor cells have also been found affected.

In new lesions the causal organism was observed only between the cells, and the conclusion had been reached that the organism was intercellular only. In later studies, with old lesions in which the tissues had broken down to some extent, the organism was found in the xylem cells, and it seemed to us to have obtained entrance into some of the parenchyma cells. The organism was subsequently found rather commonly in the xylem cells. The bacteria in the xylem cells are shown in Figs. 17, and 18, and in parenchyma cells in Fig. 19.

It would appear that the disease is primarily an infection of the parenchyma cells, but that as the dissemination of the bacteria becomes general in the parenchyma cells, infection ultimately finds its way into the xylem cells and even in some cases the phloem cells. Of the parenchyma cells, moreover, it has seemed to us that those rich in chloroplasts which lie just outside of the sheath cells surrounding the vascular bundles, have been most generally infected, and it seems probable that such cells constitute the most susceptible tissues. The infection of the xylem cells seems to explain the longitudinal spread of the reddish stripes on the leaves.

Following Erwin Smith's(2) characterization of the various types of bacterial plant diseases, this disease would seem to be comparable in type to black spot of the plum and pear blight, diseases in which the bacteria attack the parenchyma

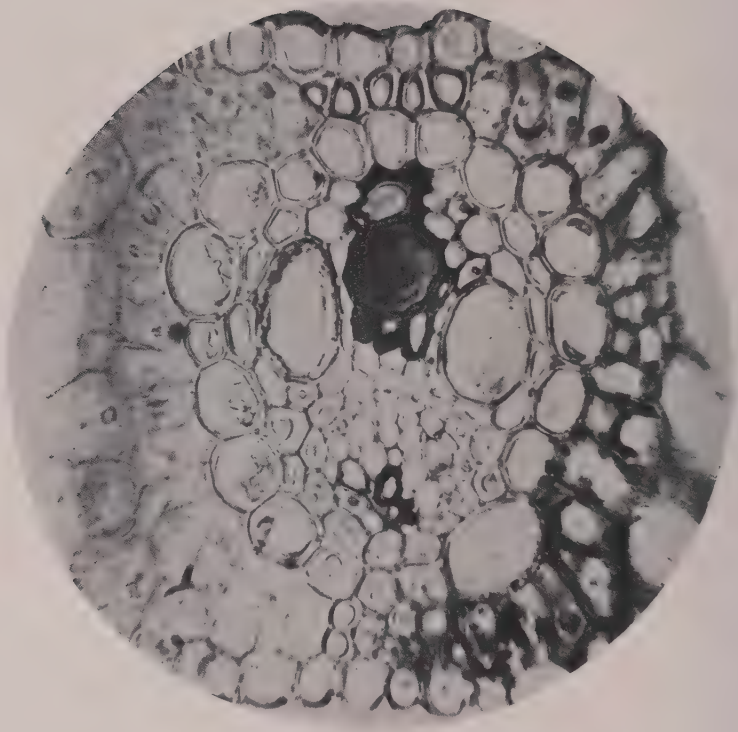

Fig. 16. This is a magnification of the vascular bundle shown in Fig. 15, X750. What seem to be bacterial masses between the cell walls of the parenchyma and in one of the xylem cells are evident. The discolored cells in the phloem are also evidence that such tissues are sometimes invaded. This lesion is of comparatively recent development.

of the leaves but do so only when the tissues are in a rapidly growing, actively dividing condition. In other words, the bacterial species causing red stripe of sugar cane is of a higher degree of parasitism than those causing rots of the parenchyma cells of storage tissues, such as Jones' carrot rot, Appel's potato rot, or Townsend's calla-lily rot. It is not of as high a type of parasitism as the bacterial species of Stewart's sweet-corn disease, or the wilt of cucurbits, both of which develop principally in the vascular system and destroy the plant by occluding the vessels.

The Effect of Bacterial Invasion on the Tissues

In sections of young leaves, unstained, the most obvious feature is the change in color of the chloroplasts from the normal green to a brownish-red color. It is this effect on the chloroplasts evidently which produces the red coloration of the long red leaf stripes. The affected chloroplasts are not disintegrated at first, but in older lesions are found broken up into smaller parts and granules, and in some lesions all trace of the chloroplasts has disappeared. In the older lesions

Fig. 17. The bacterial masses in the xylem are shown. The wall of the principal xylem cell towards the upper right-hand side seems to be dissolved to some extent, and the bacterial masses oozing out into the surrounding cells and intercellular spaces can be seen. Section stained with carbol fuchsin X750.

also the xylem cells are often observed filled with a dark, opaque, gummy substance.

There is also apparently some effect produced upon the cell walls. In the normal tissues of the sections, stained by the methods described previously, the parenchyma cell walls are of a light-blue color. At the edges of the lesions, however, the cell walls in the most recently infected tissues are stained a violet color, while the walls of the cells in the middle of the lesion are of a brownish-red color. The different reactions of these cell walls to the stains indicate that there is some action upon the cell walls by the bacteria, although the nature of the action is with us entirely a matter of conjecture. The first action, however, would seem to be on the middle lamellae, since carbol fuchsin, which has a selective action in picking out the diseased tissue, has in several new lesions picked out the middle lamellae rather clearly. Such a staining of the middle lamellae between xylem cells is shown in Fig. 20. Such action, however, apparently proceeds until the wall is so weakened that it collapses. In the photomicrograph

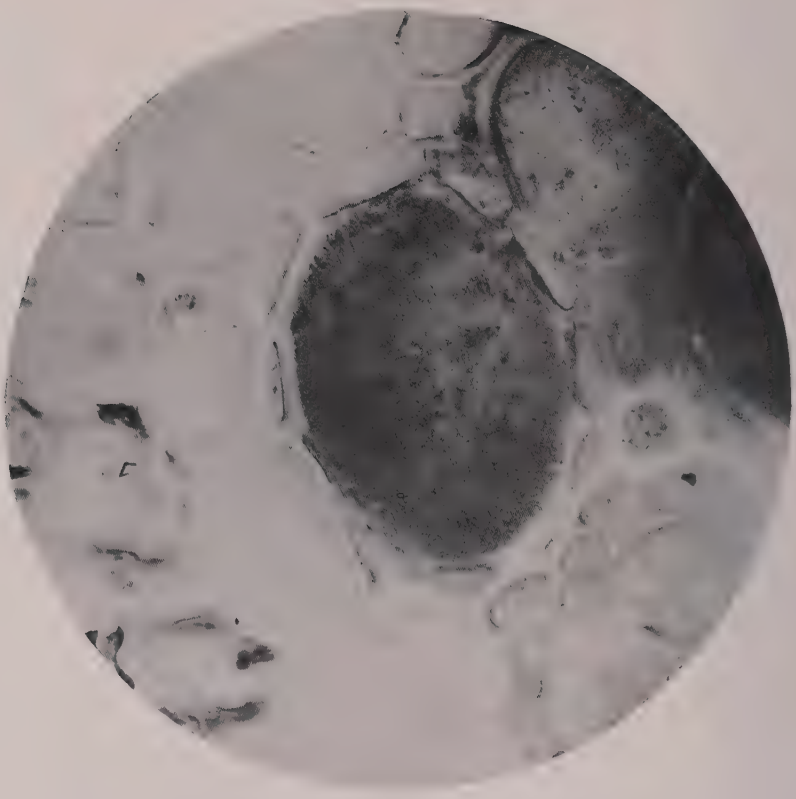

Fig. 18. The bacterial masses in the xylem cells are faintly evident. X750.

reproduced in Fig. 17, one of the xylem cells is shown with the wall very much thinner than the normal, and the bacteria apparently oozing out into the surrounding cells and intercellular spaces. In the photomicrograph in Fig. 21 the collapse of the cells resulting from the weakening of the cell walls is shown. From the observations it would appear that the injurious effects of invasion result from the by-products of the metabolism of the organism rather than the physical action of the bacteria. Such by-products must be toxic to the chloroplasts and have a lysigenic effect upon the cell walls.

The effect upon the leaf tissues as a whole is also shown in the photomicrograph in Fig. 21, the epidermis being but slightly affected, is intact, while the collapse of the parenchyma cells has led to a drawing together of the opposite leaf surfaces except in those places where the less affected sheath cells and sclerenchyma cells hold the epidermal layers apart. In some of the old lesions, even the walls of the sheath cells are discolored, although the cell contents have stained normally. There is no splitting or tearing of the tissues from this disease as in some bacterial diseases of plants, but rather a collapse of the tissues. Neither hyperplasia nor hypertrophy was observed.

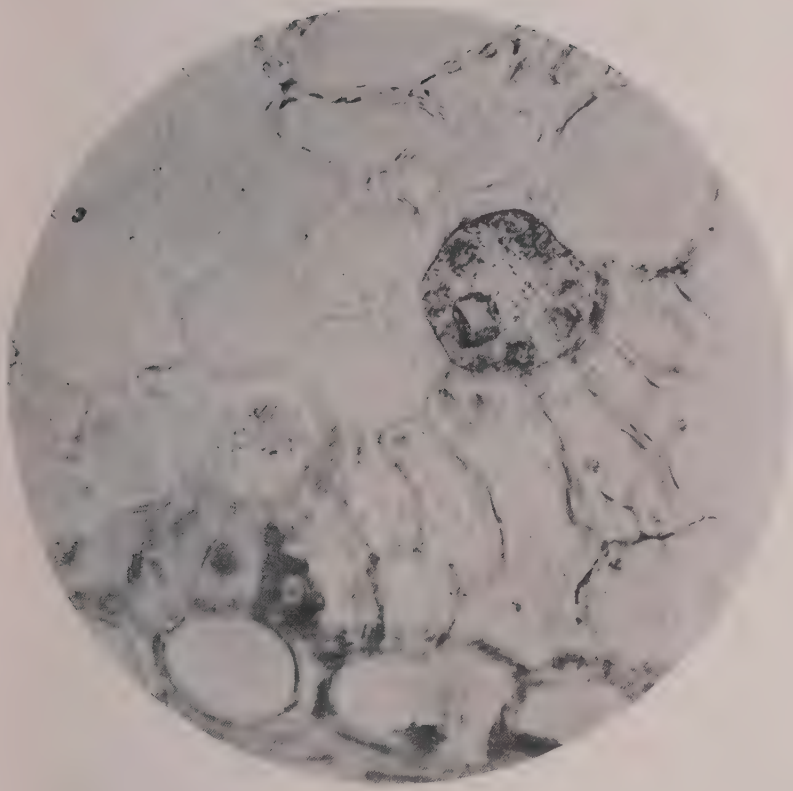

Fig. 19. Bacterial masses in parenchyma and sheath cells. X750.

The effect of the disease therefore is to destroy the chloroplasts and cause a collapse of the parenchyma tissues. In advanced cases the xylem cells of the vascular bundles are occluded. Affected tissues are entirely prevented from functioning as normal leaf tissue should. As Erwin Smith(3) has pointed out for similar diseases of other plants, this condition must result in a stunting effect, although probably not measurable in the case of this disease of sugar cane.

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(1) Smith, Erwin F. Bacteriology in Relation to Plant Diseases. Vol. II, p. 62. Carnegie Institution of Washington, D. C.; 1911.

(2) ———— Idem, p. 69.

(3) ———— An Introduction to Bacterial Diseases of Plants, p. 48. W. B. Saunders Company, Philadelphia; 1920.

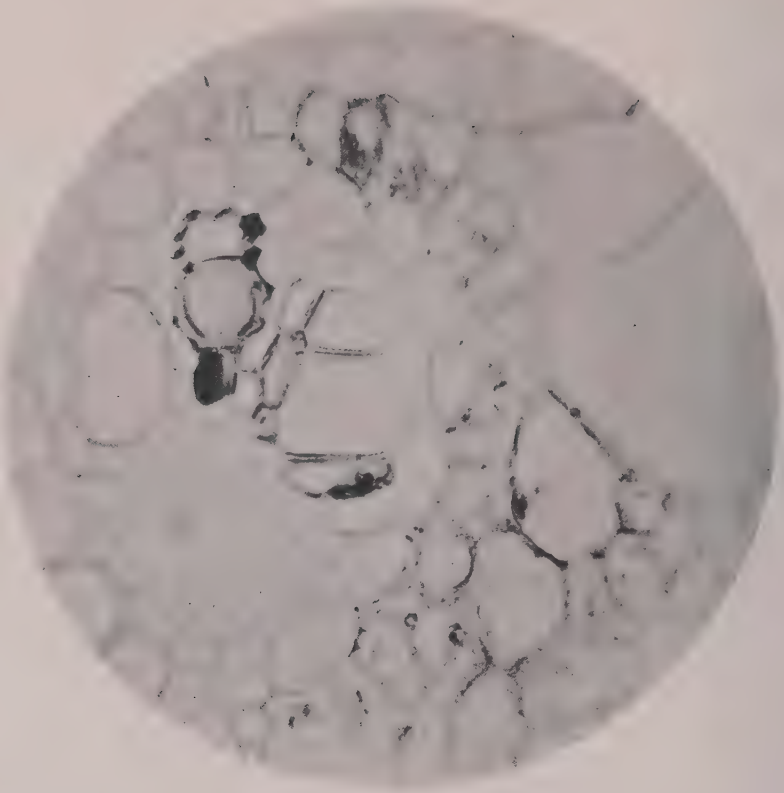

Fig. 20. Carbol fuchsin has had a selective action on the tissues affected with red-stripe disease. The way in which the middle lamellae of the cell walls has been picked out in this section, stained with carbol fuchsin, leads to the belief that the first action upon the cells leading to a collapse of the tissues is upon such middle lamellae. The magnification is X750.

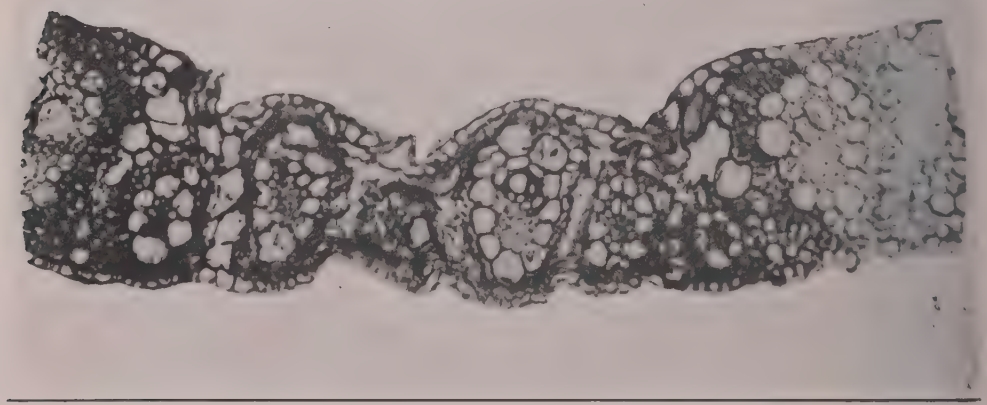

Fig. 21. At the extreme ends of the section shown in this photomicrograph are normal tissues, while the collapsed parenchyma cells in the lesions have led to a general collapse of the tissues except where the lesser affected sclerenchyma and sheath cells hold the tissues more rigidly. The less affected epidermal layer is also seen to be intact. The selective action of carbol fuchsin on the lesion is shown. This photomicrograph is about X100.

Cane Varieties Resistant to Bacterial Red-Stripe Disease

By H. Atherton Lee and Clyde C. Barnum[1]

The most effective method of combating a cane disease, if entire exclusion of the trouble has not been possible, is the use of resistant varieties. In substituting a new variety, however, it is obvious that, in addition to resistance to the disease, the variety must be equal to the susceptible cane in cane production per acre, in quality ratio, in shading-in ability, and other such important features. As an example, if a variety resistant to red stripe were substituted for the Tip canes in the Kohala district, which did not have the ability of the Tip canes to produce sugar at high elevations, then the reduced acreage, or the reduced sugar per acre is a loss caused by the disease, and the control of the disease cannot be considered entirely effective.

With this viewpoint, determinations of the degree of susceptibility or resistance of the commercially grown varieties in these islands, and the most promising of the Kohala seedlings were undertaken. The degree of susceptibility or resistance was determined by inoculation of the varieties to be tested with the bacteria of red-stripe disease, under artificially maintained conditions of moisture and environment especially favorable to the disease.

Degree of Susceptibility of Commercially Grown Varieties

The varieties D 1135, H 109, Yellow Caledonia and Badila growing under field conditions at Hawi Plantation were first inoculated. Yellow Tip cane was also inoculated for comparison. Ten plants of each variety were tested, two leaves to each plant, and five inoculations to each leaf, or 100 inoculations in all for each variety. All varieties tested were under irrigated conditions and were in good, vigorous growing condition. The plants tested were from 1½ to 3 feet high, which is a susceptible stage of growth.

Inoculations were made with needle punctures, introducing the bacterial organism from pure cultures on agar slants into the tissues of the leaves. The two youngest leaves emerging from the central cylinder were used for the test, since it was found that they were more susceptible than the older leaves. After inoculation the leaves were sprinkled with water and, in order to maintain conditions favorable for the development of the disease, moist cotton was placed at the base of the leaves and they were then inclosed in paraffin paper and wrapped with opaque news paper. These precautions were taken to maintain the proper moisture conditions and exclude the direct sunlight, which would minimize the activities of the causal bacteria. The results of the inoculations are shown in Table I.

[1] These investigations were aided by the effective cooperation of the Hawi Mill and Plantation Company, Ltd., the Kohala Sugar Company and the Union Mill Company, in whose fields the work was carried on.

Table I

Showing the Results of Inoculations With the Red-Stripe Bacteria on the Varieties D 1135, H 109, Yellow Caledonia, Badila and Striped Tip

Inoculations Made May 5, 1924; Results Examined May 17, 1924

Variety	Percentage positive	Total length of stripes Inches	Variety	Percentage positive	Total length of stripes Inches
D 1135	0	..	H 109	0	..
"	0	..	"	0	..
"	0	..	"	0	..
"	0	..	"	0	..
"	0	..	"	0	..
"	40	4*	"	0	..
"	0	..	"	0	..
"	0	..	"	0	..
"	0	..	"	0	..
"	0	..	"	0	..
Totals	4	4	Totals	0	..
Badila	0	..	Striped Tip	70†	
"	0	..	" "	..	
"	0	..	" "	100	
"	0	..	" "	70	
"	Leaf tissues killed by nitrate of soda		" "	60	
"	0	..	" "	70	
"	Leaf tissues killed by nitrate of soda		" "	70	
"	0	..	" "	80	
"	0	..	" "	80	
"	0	..	" "	70	
Totals	0	..	Totals	74	
Yellow Caledonia	0	..			
" "	0	..			
" "	0	..			
" "	0	..			
" "	0	..			
" "	0	..			
" "	0	..			
" "	60	1½*			
" "	0	..			
" "	0	..			
Totals	6	1½			

* Stripes on D 1135 and Yellow Caledonia were very narrow and short. They were negligible from a plantation viewpoint.

† No measurements were made of these stripes. The total length, however, would be well over 100 inches.

The failure to produce red-stripe disease upon inoculation with the causal bacteria on D 1135, Badila, Yellow Caledonia and H 109, while under similar conditions the bacteria produced red stripe readily on Yellow Tip cane, indicates a strong resistance of the four first-mentioned varieties. Of these, D 1135 occasionally shows short stripes, but usually very thin. It is probably more susceptible than the other three varieties. It is safe to conclude, however, that D 1135, Badila, Yellow Caledonia and H 109 are commercially resistant to red-stripe disease. Extensive field observations support these conclusions.

Susceptibility and Resistance of Kohala Seedlings

Only those canes of the Kohala seedlings which were considered promising agriculturally were tested for their degree of susceptibility to red stripe. The promising seedlings had been selected by George Watt, Manager of Kohala Plantation, and W. C. Jennings, of the Experiment Station, for their quick growth, cane-forming characters and qualities of their juices. Of these seedlings tested, Kohala 4, K 73, K 86, K 101, K 107, K 115, K 117, K 180 and K 202 are from D 1135 parentage. Manoa 198 is a Tip seedling. Kohala 382 is believed to be a seedling of H 146.

Inoculations of these seedlings were made in various fields, in the Hawi seedling field, in the Kohala seedling field, and in one of the upper fields of Union Mill Company.

First Test: The first test was made under field conditions at the Kohala Sugar Company. Inoculations with the red-stripe bacteria were made on July 14 and results observed July 28; the methods used were similar to those described for testing the susceptibility of H 109 and the other commercial varieties. One hundred inoculations were made on each variety tested and the percentages of infection follow: K 86, 51 per cent; K 101, 20 per cent; Tip cane, 15 per cent; K 73, 14 per cent; K 202, 13 per cent; K 107, 8 per cent; K 117, 3 per cent, and K 115, 1 per cent. These results were not as carefully controlled as those carried on later in the pathology nursery; however, they yield a comparison of the reactions of the Kohala seedlings to red stripe.

Second Test: In the next test, also under field conditions at the Kohala Sugar Company, a different measure of susceptibility was applied. Very often an inoculation with the red-stripe bacteria will cause a reddening of the tissue around the point of inoculation, although no definite stripe is formed. Needle punctures without the bacteria cause no such reddening but immediately heal up. The reddened lesions must, therefore, be considered positive infections, although since they do not spread into stripes in many instances they cause little or no loss of active leaf tissue and therefore cause little loss to the plant. The length of stripes formed was therefore carefully measured at each point of inoculation; this gives a much better idea of comparative susceptibility than a comparison of the percentage of positive infections.

The total lengths of all stripes produced from each 100 inoculations in this series of tests were as follows: Yellow Tip, 355 inches; K 180, 317 inches; K 101, 267 inches; K 86, 262 inches; K 117, 194 inches; K 107, 80 inches; K 382, 23 inches; K 202, 28 inches; D 1135, 24 inches; and K 115, 16 inches. Results

on K 73 in this test were not trustworthy because of the poor conditions for infection in the plants used.

Third Test: The next comparison of susceptibility following inoculations was made in the seedling field at Hawi Mill & Plantation Company, Ltd. In this test, by the same methods, the order of susceptibility was as follows: K 86, 464 inches; Yellow Tip checks, 365 inches; K 101, 197 inches; K 202, 160 inches; K 117, 135 inches; K 107, 56 inches; K 73, 48 inches; Manoa 198, 34 inches, and K 115, 23 inches. No checks on D 1135 were available in this test.

Fourth Test: This test was under the best conditions to afford a fair comparison of susceptibility, since all plants were growing under carefully controlled, equalized environment, and the conditions were made especially favorable for infection. These results in the nursery will be presented in detail, since they are the most representative. Cuttings of the varieties used in these tests were furnished by the Agricultural Department of the Experiment Station.

Inoculations on seedlings were made in the same manner as described for the tests of commercially grown varieties. As an illustration of the methods and type of results obtained, the data for the first test in the pathology nursery follow in Table II. In these tests, both K 86 and K 101 developed greater amounts of red stripe than the Tip cane; K 86 with 345 inches, and K 101 with 339 inches. These same comparative results, obtained repeatedly, have led to the conclusion that both K 86 and K 101 are more susceptible to red stripe than are the Tip canes.

TABLE II

Showing Results of Inoculations of Kohala Seedlings With the Causal Bacteria of Red-Stripe Disease

Inoculations Made August 23, 1924; Results Observed September 4, 1924

Kohala No. 4

Number of stalk	Number of stripes		Length of Stripes—Inches		Total length of stripes
	At punctures	Stomatal	At punctures	Stomatal	
1	8	..	negligible	..	....
2	9	..	"	..	....
3	8	..	"	..	....
4	8	2	"	negligible	....
5	9	..	"	..	....
6	9	2	"	negligible	....
7	9	6	"	"	....
8	8	..	"	..	....
9	7	7	"	negligible	....
10	7	..	10.5	..	10.5
Totals.....	82	17	10.5	..	10.5

Kohala No. 86

1	8	..	14.0	..	14.0
2	10	..	82.0	..	82.0
3	10	2	15.5	negligible	15.5
4	10	1	32.5	9.0	41.5
5	10	1	34.0	5.5	39.5
6	9	2	44.0	negligible	44.0
7	9	4	19.5	"	19.5
8	7	1	47.0	"	47.0
9	8	..	21.0	..	21.0
10	10	1	20.0	1.0	21.0
Totals.....	91	12	329.5	15.5	345.0

Kohala No. 101

1	10	5	93.0	7.5	100.5
2	10	2	51.5	3.5	55.0
3	9	4	1.0	6.5	7.5
4	10	2	15.0	2.5	17.5
5	9	1	21.5	9.5	31.0
6	10	3	46.0	6.0	52.0
7	9	6	17.5	5.0	22.5
8	9	1	15.5	0.5	16.0
9	9	1	3.0	1.5	4.5
10	10	3	28.0	4.5	32.5
Totals.....	95	28	292.0	47.0	339.0

Kohala No. 117

1	8	..	negligible	..	...
2	10	..	5.5	..	5.5
3	10	..	3.5	..	3.5
4	10	..	2.0	..	2.0
5	10	..	2.0	..	2.0
6	10	..	4.0	..	4.0
7	10	1	1.5	1.0	2.5
8	10	..	3.5	..	3.5
9	10	..	8.0	..	8.0
10	10	..	3.5	..	3.5
Totals.....	98	1	33.5	1	34.5

Kohala No. 107

1	8	1	13.0*	6.0	19.0
2	10	..	8.0	..	8.0
3	4*	..	10.5	..	10.5
4	9	..	1.5	..	1.5
5	4	1	5.0	0.5	5.5
6	10	..	4.0	..	4.0
7	10	..	19.5	..	19.5
8	5	1	16.5	0.5	17.0
9	4	1	4.5	0.5	5.0
10	2	1	0.5	0.5	1.0
Totals.....	66	5	83.0	8.0	91.0

Kohala No. 73

1	7	1	7.5	negligible	7.5
2	10	..	32.5	..	32.5
3	8	..	3.5	..	3.5
4	8	1	7.0	1.5	8.5
5	9	1	4.0	3.0	7.0
6	5*	1	...	3.0	3.0
7	10	..	4.0	..	4.0
8	3*	..	3.5	..	3.5
9	9	..	1.5	..	1.5
10	8	..	0.5	..	0.5
Totals.....	77	4	64.0	7.5	71.5

Kohala No. 202

1	10	2	2.5	0.5	3.0
2	9	4	1.5	8.5	10.0
3	10	1	1.5	0.5	2.0
4	10	..	6.5	..	6.5
5	10	1	13.5	17.5	31.0
6	10	1	25.0	4.0	29.0
7	10	..	26.5	..	26.5
8	10	1	20.0	0.5	20.5
9	10	..	18.0	..	18.0
10	10	..	6.0	..	6.0
Totals.....	99	10	121.0	31.5	152.5

Kohala No. 115

1	5	..	0.5	..	0.5
2	8	1	negligible	0.5	0.5
3	10	..	0.5	..	0.5
4	8	..	0.5	..	0.5
5	10	..	negligible	..	...
6	9	1	0.5	0.5	1.0
7	8	..	6.0	..	6.0
8	10	..	negligible	..	...
9	10	1	"	1.0	1.0
10	9	..	"	..	...
Totals.....	77	3	8.0	2.0	10.0

Striped Tip as Controls

1	10	..	8.5	..	8.5
2	10	2	13.5	24.0	37.5
3	10	2	69.5	14.0	83.5
4	10	1	11.0	10.0	21.0
5	10	..	39.5	...	39.5
6	10	1	31.0	5.5	36.5
7	10	..	1.5	...	1.5
8	10	..	30.5	...	30.5
9	10	..	0.5	...	0.5
10	10	..	7.0	...	7.0
Totals.....	100	6	212.5	53.5	266.0

* 1 leaf decayed.

D 1135 as Controls

1	10	1*	4.0	negligible	4.0
2	10	..	3.0	...	3.0
3	10	..	2.5	...	2.5
4	10	..	2.5	...	2.5
5	9	1	3.0	6.0	9.0
6	8	..	3.0	...	3.0
7	10	..	...	...	...
8	8	1	3.0	1.0	4.0
9	9	..	0.5	...	0.5
10	9	..	2.0	...	2.0
Totals.....	93	3	23.5	7.0	30.5

On the other hand, K 115 developed only 77 per cent positive infections as compared with 93 per cent for D 1135, and the length of stripes was only 10 inches as compared to 30 inches for D 1135, and 266 inches for Striped Tip. These results, obtained repeatedly, lead to the conclusion that K 115 is even more resistant than D 1135. K 4 also developed only 10 inches of red stripe from 100 inoculations. K 117 developed 34 inches of red stripe from the same number of inoculations; there was only one stomatal infection on this variety. K 73, K 107 and K 202 all developed much less red stripe than Striped Tip cane; and K 73, at least, approaches very close to commercial resistance.

Fifth Test: This last test, also in the pathology nursery at Hawi, gave comparable results to the previous tests. The extent of invasion of the leaf tissue was as follows: K 101, 243 inches; K 86, 216 inches; Striped Tip, 181 inches; K 117, 74 inches; D 1135, 56 inches; K 73, 41 inches; K 107, 41 inches; K 4, 38 inches; K 115, 21 inches, and K 202, 11 inches. The results of all five tests have been summarized and are shown in Table III. From these five series of inoculations, the results of which agree very closely, the following conclusions seem warranted: Kohala 115 is more resistant to red stripe than D 1135. K 73, K 107 and K 117 are but slightly more susceptible than D 1135; they can be safely considered as commercially resistant. K 202 is slightly more susceptible, but is not nearly as susceptible as the Tip canes. Red-stripe disease would occur to a slight extent in fields of 202, but would probably be much less severe than in fields of Tip canes under same conditions. Manoa 198 has promise of being quite resistant. K 86 and K 101 can be discarded, since they are considerably more susceptible than the Tip canes. K 4, although possessing some degree of resistance to red stripe, is not considered a good cane by the agriculturists. Although there was only one test of K 382, the conditions of the test were entirely satisfactory and the indications of a considerable degree of resistance of this variety are well warranted. K 382 is regarded as a very promising cane for the lower fields by George Watt.

* The stripes which occur on D 1135 are very narrow instead of broad as on leaves of Tip canes.

TABLE III

Summary of the Results of All Inoculations to Test the Comparative Susceptibility of Seedling Varieties at Kohala

Variety	No. of test	No. of inocula-tions	Percentage of stripes at inoculations	No. of stomatal infections	Length of all stripes in inches	Average length of stripes per stalk
Kohala 86.....	1	100	51	..	...	
"	2	100	47	..	262	
"	3	100	68	..	464	
"	4	100	91	12	345	
"	5	100	95	7	216	
Total & Avgs....	5	500	70	19	1287	32.2
K 101	1	100	20	..	...	
"	2	100	36	..	267	
"	3	100	60	..	197	
"	4	100	95	28	339	
"	5	100	96	1	243	
Total & Avgs....	5	500	61	29	1046	26.2
Tip canes.....	1	100	15	..	...	
"	2	100	80	..	355	
"	3	100	91	..	365	
"	4	100	100	6	266	
"	5	100	93	2	181	
Total & Avgs....	5	500	76	8	1167	29.2
K 202.....	1	100	13	..	...	
"	2	100	16	..	28	
"	3	100	32	..	160	
"	4	100	99	10	152	
"	5	100	95	1	11	
Total & Avgs....	5	500	51	11	351	8.8
K 73	1	100	14	..	...	
"	3	100	33	..	48	
"	4	100	77	4	71	
"	5	100	79	0	41	
Total & Avgs....	4	400	51	4	160	5.3
K 107	1	100	8	..	...	
"	2	100	28	..	80	
"	3	100	52	..	56	
"	4	100	66	5	91	
"	5	100	87	9	41	
Total & Avgs....	5	500	46	14	268	6.7

K 117.....	1	100	3	..	...	
"	2	100	56	..	194	
"	3	100	59	..	135	
"	4	100	98	1	34	
"	5	100	93	4	74	
Total & Avgs....	5	500	62	5	437	10.9
D 1135.....	2	100	9	..	24	
"	4	100	93	3	30	
"	5	100	77	3	56	
Total & Avgs....	3	300	60	6	110	3.7
K 115.....	1	100	1	..	...	
"	2	100	5	..	16	
"	3	100	17	..	23	
"	4	100	77	3	10	
"	5	100	90	11	21	
Total & Avgs....	5	500	38	14	70	1.7
K 4.....	4	100	82	17	10	
"	5	100	82	0	38	
Total & Avgs....	2	200	82	17	48	2.4
K 382.....	3	100	8	..	23	2.3
Manoa 198.....	3	100	40	..	34	
"	6	100	88	0	2	
Total & Avgs....	2	200	64	0	36	1.8

Field Tests

In order to check the results from artificial inoculations with the amount of infection from natural sources under natural conditions, a planting of the seedling varieties was made in one of the upper fields of Homestead Plantation, one of the holdings of the Hawi Mill and Plantation Company, Ltd., where infection was abundant. In a field of young plant cane of the Yellow Tip variety, alternate rows were dug up for a length of 30 feet and replanted, one line to each of the Kohala seedlings. A line of each of Uba numbers 1, 2, 3, 4 and 5, and one line each of Striped Tip and D 1135 were also planted. The lines of young Tip cane alternating with the lines of seedlings thus afforded abundant sources for natural infection. The results showed a remarkable correlation with the results of artificial inoculations. K 101 and K 86 showed fully as much infection as the Tip cane, if not more so. Kohala 202 was considerably less susceptible than Tip cane, although it showed red stripes; this variety does not suffer from red stripe as do the young Tip canes. It would be very difficult to say, from the tests reported here, which was the most resistant of K 117, K 107 and K 73; all three may be safely considered commercially resistant.

K 115 showed even less red stripe than D 1135, one line of which was planted as a control on the Kohala seedling varieties. K 4 also showed a high degree of resistance. The Uba hybrids Nos. 1, 2, 3, 4 and 5 all showed commercial resistance to the disease. Uba 1 showed a few red stripes when very young, but can be considered sufficiently resistant for plantation use without material loss from red stripe. Of interest, in passing, are indications of a considerable degree of resistance to mosaic disease by K 73. It is considered as a cane for upland fields only, however, since under conditions favorable for tasseling it tassels profusely.

There are no variety tests to show comparative sugar yields for these varieties yet. George Watt and W. C. Jennings, who have worked a great deal with the Kohala seedling varieties, however, feel very optimistic concerning K 202 and K 107. Mr. Watt thinks K 115 has considerable promise also; he finds that it does not tassel to any extent, and states that it appears to be resistant to drought and wind. From our contact with this cane, in the resistance to red-stripe tests it can be said that K 115 is very much like D 1135 in many ways, and, in addition, shades in the rows much more quickly than D 1135. Manoa 198 is also regarded as very promising by the agriculturists.

Of the seedlings discussed in this report, K 73, K 107, K 115, K 117, K 202, K 101 and K 86 are of D 1135 pistillate parentage. K 4 and K 382 are seedlings of H 146. Manoa 198 is a seedling of Yellow Tip. It is rather difficult to formulate any correlations between parentage in these seedlings and their susceptibility to red-stripe disease.

Susceptibility of Native Hawaiian Varieties

A collection of native Hawaiian varieties has been brought together by Mr. Jennings at Hawi Plantation in a field where sources for infection with red-stripe disease were present. The variety known as Kea showed heavy infection from natural sources. The variety Laukona similarly showed great susceptibility. Other native varieties in the same planting showed susceptibility, although to much lesser degrees. The idea has been advanced by Caum and others, based on morphological characters, that the Tip canes, Yellow Tip, Striped Tip and Red Tip are closely related to the native Hawaiian canes. This susceptibility of native canes to red-stripe disease, comparable to the susceptibility of the Tip varieties, correlates with the suggested relationships.

Summary

(1) Artificial inoculations were made with the red-stripe bacteria on the promising Kohala seedlings selected by the agriculturists, to test for resistance or susceptibility of these varieties to red stripe. Ten plants of each seedling variety, with ten inoculations to each plant, were tried out in each series of tests. In the case of most of the varieties, these series of tests were repeated four times, making five series of tests in all.

(2) From these series of tests K 86 averaged 32.2 inches of red stripe per plant; K 101, 26.2 inches; Tip canes, 29.2 inches; K 117, 10.9 inches; K 202, 8.8 inches; K 107, 6.7 inches; K 73, 5.3 inches; D 1135, 3.7 inches, and K 115,

1.7 inches. From the results of only one or two series of tests, K 4 averaged 2.4 inches per plant; K 382, 2.3 inches; and Manoa 198, 1.8 inches.

(3) These results have been supported with plantings of these seedling varieties alternating with rows of heavily infected Tip canes, affording natural sources for infection under natural growing conditions. The results of these field tests agree very closely with the results of artificial inoculations. K 86 and K 101 are fully as susceptible as the Tip canes if not more so. K 202 has some degree of susceptibility but could be grown with but little loss from red stripe as compared to the Tip canes. K 117, K 107 and K 73 are commercially resistant to red stripe. K 115 is fully as resistant to the disease as D 1135. Manoa 198 and K 382 have indications of commercial resistance.

(4) Results of variety tests to compare the sugar production of these seedling varieties with the Tip canes are not available yet. Should these resistant seedling varieties not be comparable to the Tip canes in production, new seedling varieties can be quickly tested for their susceptibility or resistance to red stripe.

Methods of Combating Red-Stripe Disease

By H. Atherton Lee, Clyde C. Barnum and W. C. Jennings

Numerous plantation inspections have shown that red-stripe disease is at present confined to the Kohala district and has not reached other cane-growing regions of the Islands, with one exception. This was a small outbreak on Oahu in Tip cane in 1923, which was entirely eradicated.

There is little immediate concern regarding the disease on the islands of Maui and Oahu, because there are very few areas on these islands growing the Tip canes and such areas are very small. On the other hand, entire absence of the trouble is desirable even on these islands, since, in the development of new seedlings, serious susceptibility to the disease of an otherwise promising variety would necessitate the throwing out of such a variety. Quarantine restrictions which would be necessary if the disease occurred on Oahu or Maui, also would be a great handicap in the distribution of seed cuttings of new seedling varieties.

The most immediate concern, however, is in preventing the spread of the trouble into the Hamakua district, and especially into the Hilo district. These districts, according to the 1924 cane-variety census, are growing 3,333 acres of the Tip canes in the 1925 crop and fully as much in the 1926 crop. Moreover, experience with red stripe in the Kohala district has shown that it is primarily a wet-weather disease. This leads to the belief that red stripe would be a more serious trouble in the Hilo district, with its abundant, consistent rainfall, than it is in the Kohala district. The Hamakua district is also usually a region of greater rainfall than the Kohala district, although not to such an extent as the region around Hilo.

Measures for combating red-stripe disease, therefore, resolve themselves into two principal methods, the more important of which is the prevention of the spread of the trouble from Kohala into districts now free of the trouble; and secondly, the minimizing of the trouble in Kohala.

Prevention of Spread from Kohala

Possible Methods of Dissemination: Spread of the disease from Kohala is possible by infected cane cuttings, by farm implements, cane knives, pocket knives, or by indirect transmission in wet weather, such as on the clothes of men working in infected cane. A plant quarantine regulation by the Territorial Board of Agriculture and Forestry prohibits the transportation of cane cuttings from Kohala, and the knowledge of the disease possessed by the plantation men will safeguard against the spread of the disease by parts of infected cane plants.

Cultivation implements, cane knives, pocket knives, and even an automobile crushing diseased leaf tissues, in a chain of favorable circumstances, could transmit the diseases from affected fields in Kohala to healthy cane fields in Hamakua or Hilo. In the case of a bacterial disease of citrus trees in Florida, an ice wagon, rubbing against an infected dooryard tree in one district, is known to have transmitted the disease to a previously healthy orchard ten or twelve miles away. By analogy, a similar possibility exists in transmitting red-stripe disease. The results of Barnum's findings, showing that the causal bacteria of red stripe can persist in the soil for 20 or 30 days and still remain viable, would indicate that mud from an infected cane field, if carried to a non-infected field of Tip cane in Hamakua, on automobiles, shoes or field implements, could serve as a source for infection.

However, some of the characters of the red-stripe organism are unfavorable for its transmission over long distances; the causal bacteria are very readily killed by direct sunlight and by drying. In the thirty miles of windy drive between Kohala and Hamakua the mortality of chance bacteria on automobile mud guards, running boards and tires would be high. The possibilities are greater for cane men, working in the cane fields than from those of other individuals. It is advisable, therefore, for cane men, in periods of rainy weather, to refrain from driving automobiles from fields of Tip cane in Kohala to fields of Tip cane in Hamakua or Hilo. Cane knives, pocket knives, field implements and other possible carriers should certainly be disinfected if they are to be used in fields in Hamakua or Hilo after use in Kohala.

For the few research men who are working in intimate contact with the disease in Kohala, disinfection of shoes, leggings, hats and hands and arms has been practiced and is advisable in the future. The laundering or steam cleaning of clothes will certainly minimize the possibilities of spread of the disease by such means. These precautions are in the main necessary only for Experiment Station men or others who come in close contact with infected materials.

The susceptibility of the common grasses related to cane which occur in Kohala has been shown to be extremely slight. The possibility of the spread of red stripe into non-infected districts by such grasses, therefore, seems to be negligible.

There are some indications that chewing insects are agents in transmitting infection from diseased to healthy plants. The possibility of insects transmitting the disease into the Hamakua or Hilo districts must, therefore, be considered. The susceptible Tip canes at Kukuihaele are but 12 miles distant by direct air line from the nearest infected canes in the Kohala district. Of considerable advantage, however, is the extremely rough nature and uncultivated and uninhabited condition of the intervening mountains and canyons. The usual prevailing winds, moreover, are from Hamakua towards Kohala, which is an additional natural barrier.

The possibility of chewing insects in Kohala alighting in automobiles and, by a chain of circumstances, being translocated to susceptible fields in unaffected localities is, perhaps, a greater possibility than the direct flight of an insect from Kohala to Hamakua. Research men, and possibly some plantation men, might possibly take some measures against this, but drivers of rent cars and men not in touch with plantation problems would not be expected to exert any precautions against such things.

Birds are also possible carriers of the disease organism on their feet or bills.

Eradication for Outbreaks in Previously Uninfected Districts

There are, therefore, several possibilities for the spread of the disease which cannot be guarded against in any practical way. It is a matter to be expected, then, that in twenty or ten or even five years or less an outbreak of red-stripe disease will be found in Hamakua or Hilo. Such an outbreak would probably be noticeable first in fields of young cane but a foot or two in height. In the event of such a contingency, eradication of the affected cane would be the logical procedure in an area of five to ten or even twenty acres. The loss of such an area of young Tip cane would be less than the annual loss if the disease became widespread in the Hilo and Hamakua districts, and would be much less than the repeated annual loss over a period of five or ten years. In the event of need for eradication it would seem that the costs of eradication and loss of an area of cane should be shared by all plantations growing Tip canes in the Hilo and Hamakua districts, rather than by the one plantation on which the affected cane should be found. The operations of eradication should be undertaken by the Experiment Station.

With the development of resistant seedlings of agricultural value in the Kohala district, the need for quarantine precautions can be somewhat lessened. Even at the present time reserve plantings of D 1135 and the resistant Kohala seedlings, which are obtainable from the Experiment Station in Honolulu, would seem to be desirable on those plantations in Hamakua and Hilo growing large areas of Tip canes.

Measures to Minimize the Disease in Kohala

Agricultural Practices: In discussing matters of field methods it is appreciated that the advantages gained by minimizing such a minor disease as red stripe are sometimes outweighed by other considerations; for this reason it is

necessary to qualify the substance of the following paragraphs by the words "when feasible." In many instances, however, it may be quite advantageous to slightly alter practices so that red-stripe disease may be to some extent avoided and minimized.

Factors Favoring Infection: In the studies in Kohala it has developed that there are factors which are favorable to infection. It has been shown by inoculation experiments that young, actively growing leaf tissues are slightly more susceptible than the older, more mature leaves. It has also been shown by inoculation tests and field experience, that young cane is much more susceptible than old cane; cane five to six feet above the ground is scarcely affected by red-stripe disease. From the inoculation experiments, as well as by analogy with other bacterial plant diseases, it is evident that the red-stripe-disease bacteria are disseminated by hard, splashing rains accompanied by winds; moist, cloudy weather also favors the development of the disease. Infection may take place through the stomata of the leaves; stomatal infections have been readily obtained in the inoculation experiments. Infection takes place even more readily at wounds and abrasions of the leaf surfaces. Wind-whipping of the cane leaves therefore favors infection.

Suggested Field Methods: With the conditions favorable for infection well established, it is evident that if the young cane, which is the most susceptible stage for infection, can be forced through to four or five feet in height, during the drier season from April to October, losses will be minimized. Practices which favor such quick development are early planting and early application of fertilizers. Both of these practices are recommended by the Agricultural Department of this Station, independently of the effect on red stripe, because they are considered good agriculture; the rows are shaded in before the winter months of slow growth, and cultivation costs are thus lessened. The use of fertilizers late in the season is conversely favorable for red-stripe disease injury, since it pushes out new succulent growth at the time when splashing rains and long periods of cloudy, wet weather favorable for infection may be expected. Fertilizer applications, after the wet winter period favorable for red stripe, aid in minimizing the effects on the leaves by pushing out new, healthy leaves quickly to take the place of the infected leaves whose functioning area has been reduced.

Windbreaks, where feasible, lessen abrasions and wounds on the leaves and also lessen the dissemination of the causal bacteria, and so contribute also to a slight extent in lessening red-stripe infection.

The practice of cross-harrowing at right angles to the rows when the cane is just above ground results in many abrasions of the leaves, and the harrows also transmit infection directly from plant to plant. If cross-harrowing is necessary it should be done before the cane is above the ground.

It is already evident in fields in the Kohala district, where early planting and early application of fertilizers have been practiced, that these measures alone will greatly minimize the injuries due to the disease.

The Use of Resistant Varieties: The use of resistant varieties is, of course, the most effective control possible and is the most economical if the resistant varieties produce cane of equal tonnage to the susceptible varieties and have as

good quality ratios, shading-in ability and other characters for cheap production, harvesting and milling. In the selection of varieties resistant to red-stripe disease for the Kohala district, such varieties must also be adapted to cultivation at high elevations.

There are considerable areas of Tip canes being grown at the lower elevations where D 1135 can be grown advantageously from the standpoint of sugar production. The substitution of D 1135 at these lower elevations not only would entirely eliminate the losses from red-stripe disease in the lower fields but would lessen the menace of mosaic disease. The Agricultural Department of this Experiment Station states that D 1135 can be advantageously substituted for Tip cane up to an elevation of 1,200 feet; in the Hamakua district, D 1135 is being grown successfully even at elevations of 1,500 feet.

Above elevations of 1,200 feet the areas planted to the seedling varieties Kohala 115, K 107, K 117, K 73 and K 202, which have been shown to be much less susceptible than the Tip canes, could advantageously be expanded until variety tests and plantation experience have proved their agricultural value. If none of these less susceptible varieties prove of agricultural value, further susceptibility tests will undoubtedly indicate additional resistant varieties in the more recent propagations of seedlings of Yellow Tip and D 1135 parentage. Elimination of the disease by eradication in the Kohala district does not seem feasible now since infection is so widespread that almost all fields of Tip canes would have to be plowed out.

Losses from Red-Stripe Disease

Red stripe has been shown by inoculation studies to affect the mature cane stalk very slightly; the cane tops, however, where sugars are in the form of hexoses rather than sucrose, are sometimes severely affected, resulting in top rot. Leaf sheaths are susceptible, although they are seldom seriously affected. Cane roots are very susceptible and easily killed when infected by artificial inoculations. There is no evidence to indicate that root infections occur seriously to any extent naturally in the Kohala district at the present time.

Injury to the cane from red stripe results chiefly from loss of functioning leaf area; this injury is negligible in cane approaching maturity but is more appreciable in younger stages of growth. Losses are also more severe in young ratoon cane than in young plant cane. It is impossible to accurately estimate the injury resulting from losses of leaf area due to red stripe, but it is conservative to say that in young ratoon cane under upland conditions during rainy periods the loss of actively functioning leaf area may amount to 50 per cent of the total leaf surface over a period of one or two months. The loss to the crop over a period of 20 to 24 months, however, even in the most severe cases seen in Kohala to the present time, could not exceed 10 per cent of the crop, and under the usual lowland field conditions losses seem to be less than 1 per cent of the total crop.

These estimates for the total losses include losses resulting from top rot. Red-stripe infection in cane tops results in the formation of lalas; these affected canes at first are prematurely ripened and give at first better juices, but with

time, deterioration results and the juice qualities become much poorer. In one field as high as 10 per cent of the cane sticks showed top rot, but such high infection is unusual; the usual number would be less than one per cent in the Kohala district. It would be expected that top rot resulting from red stripe would be much greater if the disease became established in the Hilo district, where rainfall is much more abundant, and under these conditions the losses there would be greater.

When it is considered that these losses from red stripe occur only on the Tip canes, and that red stripe on Tip canes is confined to the 3,000 acres of Tip canes in the Kohala district, it is appreciated that the actual loss to the Territory as a whole is not great as yet; moreover red stripe is not entirely distributed throughout the Kohala district at the present time.

Summary

(1) Red-stripe disease is at present confined to the Kohala district and has not been found in the Hamakua or Hilo districts on Hawaii, or on the other islands. Every effort to prevent the spread of the trouble from Kohala to unaffected districts is necessary.

(2) The transportation of cane cuttings from Kohala is inadvisable and is prohibited by Territorial quarantine regulations. Field implements used in Kohala should not be used in other districts unless adequate disinfection is given them. Pocket knives, cane knives and other possible carriers used in Kohala should also be disinfected. Cane men, and especially Experiment Station men who may be in intimate contact with the disease in Kohala, should disinfect their shoes, leggings, hands and hats if they are subsequently to be engaged in work in fields of Tip cane elsewhere. The laundering or steam cleaning of previously infected clothing will minimize chances of infection by contact with susceptible cane.

(3) The possibility exists of the spread of infection to districts at present unaffected, in the course of the next 20 or 10 or even 5 or less years, due to dissemination by chewing insects, birds, or by the carelessness of men working in infected fields. In the event of such an outbreak, the cost of eradication of the diseased cane in newly affected areas would be less than the annual losses from the disease throughout the whole newly infected district, and much less than the annual losses repeated year after year. Eradication work should be undertaken by the Experiment Station.

(4) Agricultural practices may aid in minimizing the disease in Kohala. It is evident that red stripe is primarily a disease of young cane, and is also a wet-weather disease. With this in mind, it is possible and has been shown by practice that early planting and early applications of fertilizers can be made to force the cane through to a height of 3 or more feet before the winter rains may be expected. In other words, efforts should be made to get the cane through its susceptible stage, that is, when it is young, during the drier summer weather.

(5) The control of the disease by resistant varieties would be the most effective and economical, if such resistant varieties can be grown as cheaply and will yield as well as the Tip canes at high elevations. With these points in view, the most promising Kohala seedlings have been tested out to determine their sus-

ceptibility or resistance; five of these seedling varieties have been shown to be much less susceptible than the Tip canes. Variety tests are to be undertaken by the Agricultural Department to compare yields from these resistant seedlings with yields from Tip canes.

(6) Losses to these Islands as a whole are not great at the present time, since red stripe affects only the Tip varieties and is moreover confined to the Kohala district. Losses in Kohala on Tip canes are due to decreased functioning leaf area, which is considerable when the cane is young, and to top rot resulting from the disease when the cane is older. The upper fields of more rainfall usually show heavy leaf infection and top rot much more than the lower fields. Accurate figures of the losses due to the disease are impossible to determine at reasonable expense, but estimates would place the decrease in crop between 10 per cent on the most seriously infected fields, which are few in number, to less than 1 per cent on the lower fields. Since red stripe is a wet-weather trouble, in case the disease should become established in the Hilo district, losses would be expected to be greater there than in Kohala.